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SCIENTIFIC DATA REVIEWS
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MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

07-DEC-1998

SUBJECT: PP#7F04882. Pyriproxyfen in/on Pome Fruits and Walnuts. Evaluation of Residue Data and Analytical Methods. Chemical 129032. Barcode D238190. Case 289048. Submission S528153. MRID #s 44329505 thru 44329512.

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The following is a review of pyriproxyfen residue chemistry data submitted in support of a Section 3 permanent registration for pome fruits and walnuts. The initial review was conducted by Dynamac under the supervision of HED. The Dynamac review has undergone secondary review in RAB1 and has been revised to reflect current policies and decisions.

Executive Summary of Chemistry Deficiencies

- Revised KNACK™ label with specification of ground or aerial application equipment clearly indicated under Special Instructions for each pest use for apples, pears, and walnuts.
- Revised KNACK™ label with specification of amount of spray volume clearly indicated under Special Instructions for each pest use for apples, pears, and walnuts.
- Agency validation of analytical method for apples and walnuts.

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PYRIPROXYFEN

PERMANENT TOLERANCE PETITION (PP#7F04882) FOR USE OF PYRIPROXYFEN ON POME FRUITS AND WALNUTS

INTRODUCTION

Valent USA Corporation has submitted a petition for the establishment of permanent tolerances for residues of the insecticide pyriproxyfen in conjunction with a request for an amended Section 3 registration of an 0.86 lb ai/gal emulsifiable concentrate (product name: KNACK™ Insect Growth Regulator) for use on pome fruits and walnuts. The petitioner is proposing the establishment of permanent tolerances for residues of pyriproxyfen *per se* as follows:

Pome fruits	0.2 ppm
Walnuts	0.02 ppm
Apple pomace, wet	0.8 ppm

Pyriproxyfen [2-[1-methyl-2-(4-phenoxyphenoxy)ethoxy]pyridine] is an analogue of insect juvenile hormone which interferes with the hormonal control of insect growth and development, thereby inhibiting egg hatch, larval embryogenesis, metamorphosis, and adult emergence. The 0.86 lb ai/gal EC was developed for use in insect resistance management (IRM) and integrated pest management (IPM) programs.

Permanent tolerances for pyriproxyfen have been established under 40 CFR 180.534 at 0.05 and 2.0 ppm on cotton seed and cotton gin byproducts, respectively (PP#6F04737, D241303 & D228499, W. Donovan, W. Dykstra, and B. Tarplee, 27-FEB-1998). Previous to the cotton petition, pyriproxyfen was registered for only non-food uses. Based on plant metabolism studies conducted on cotton, apple, and tomato, the HED metabolism committee has determined that the residue of concern in plants is pyriproxyfen *per se* (D250953, W. Donovan & W. Dykstra, 19-NOV-1998).

Associated with this petition are 8 volumes of residue chemistry submissions which are evaluated in this document.

CONCLUSIONS

OPPTS GLN 860.1200: Proposed Uses

1. The proposed use directions for the 0.86 lb ai/gal emulsifiable concentrate formulation on apples, pears, and walnuts are inadequate. Information concerning application equipment (i.e., ground or aerial equipment and amount of diluent per acre) should be included on the proposed label. A revised Section B should be submitted.

OPPTS GLN 860.1300: Nature of the Residue - Plants

2a. Apples were treated with pyridyl- and phenyl-labeled [^{14}C]pyriproxyfen at 1.2x the maximum seasonal rate and harvested 45 days following the last of three applications. The parent pyriproxyfen was the major component of the residue present at 52-54% of the TRR (0.097-0.101 ppm). The second most prevalent metabolite identified was metabolite 4'-OH-PYR, present at 9-11% (0.017-0.021 ppm). Metabolism of pyriproxyfen in apples proceeds through hydroxylation and cleavage of the phenoxy ether linkage. Primary metabolites formed are further metabolized to more polar products by oxidation or conjugation reactions. Similar metabolic pathways were observed for the metabolism of pyriproxyfen in cotton, goats, and hens.

2b. The nature of the residue in plants is adequately understood. The HED Metabolism Assessment Review Committee (MARC) determined that the residue of concern in pome fruits and walnuts is pyriproxyfen *per se* (D250953, W. Donovan & W. Dykstra, 19-NOV-1998).

OPPTS GLN 860.1300: Nature of the Residue - Animals

3a. No animal metabolism data were submitted with this petition. Ruminant and poultry metabolism studies have previously been submitted and reviewed (PP#6F04737, DP Barcode D228556, J. Garbus, 06-MAY-1997) in conjunction with a petition for cotton. The animal metabolism studies demonstrated that the transfer of ^{14}C residues to tissues was low. Total residues in goat milk, muscle, and tissues accounted for less than 2% of the TRR. Total residues in poultry eggs, muscle, and tissue accounted for ~2.7% of the TRR.

3b. The nature of the residue in animals is adequately understood. The HED MARC determined that should future crop uses increase the maximum theoretical dietary burden to the point that tolerances are needed in animal commodities, the residue of concern will be pyriproxyfen and the free and sulfate forms of 4'-OH-PYR (D250953, W. Donovan & W. Dykstra, 19-NOV-1998).

OPPTS GLN 860.1340: Residue Analytical Methods

4a. The GC/NPD and HPLC methods RM-33P-1-3 and RM-33N-2 are adequate for the purposes of collection of residue data for pyriproxyfen and 4'-OH-PYR residues in/on apple, processed apple, pear, and walnut commodities. The LOQ is 0.02 ppm for each

analyte. **A conclusion on the adequacy of the methods for enforcement of permanent tolerances will be withheld pending a satisfactory agency petition method validation (PMV) of methods RM-33P-1-3 and RM-33N-2 for apples and walnuts, respectively.**

4b. Independent laboratory validation data have been submitted for Method RM-33P-1-3 on apples. HED concludes that the ILV was successful for the analysis of pyriproxyfen and 4'-OH-PYR.

4c. Method RM-33-P-1-3 has been adequately radiovalidated for pyriproxyfen and 4'-OH-PYR, using samples of apple pomace from the [^{14}C]pyriproxyfen apple metabolism study.

4d. Residues of pyriproxyfen and its metabolites, 4'-OH-PYR, POP, and 2,5-OH-pyridine in animal tissues were analyzed using GC/NPD and HPLC Methods RM-33G-2 (pyriproxyfen, 4'-OH-PYR, and POP in milk), RM-33G-3 (2,5-OH-pyridine in milk), RM-33T-1 (pyriproxyfen in tissues), RM-33T-2 (4'-OH-PYR in tissues), RM-33T-3 (POP in liver and kidney), and RM-33T-4 (2,5-OH-pyridine in liver and kidney). These methods are adequate for data collection. LOQs were 0.02 ppm for each analyte, although interferences increased the LOQ to 0.04 ppm for metabolites in some matrices.

4e. Samples from a goat metabolism study (PP#6F04737, DP Barcode D228556, J. Garbus, 06-MAY-1997) were re-analyzed using Methods RM-33G-2 and RM-33G-3 for milk, and Methods RM-33T-1, RM-33T-2, and RM-33T-4 for liver. The results from the data collection method were in good agreement with those from metabolite analysis.

OPPTS GLN 860.1360: Multiresidue Method

4f. Multiresidue testing data have previously been provided (PP#6F04737, DP Barcode D228556, J. Garbus, 06-MAY-1997) for pyriproxyfen and its metabolite PYPAC. Pyriproxyfen was recovered from fortified apple and cotton samples through protocol A, C, D, E, and F. The metabolite PYPAC was tested with protocols A, B, C, and D. The results have been forwarded to FDA.

OPPTS GLN 860.1380: Storage Stability Data

5a. Residues of pyriproxyfen are stable in frozen storage conditions for at least 3 months in apples and walnuts, and 2 months in apple pomace. The samples from the residue studies were stored for a maximum of 85, 38, and 57 days for apples and pears, walnuts, and apple pomace, respectively. HED concludes that storage stability has been demonstrated for the purposes of this petition.

5b. Residues of pyriproxyfen are stable in frozen storage conditions for at least 30 days in cow liver, fat, and muscle. The samples from the ruminant feeding study were stored for

less than 1 month. HED concludes that storage stability has been demonstrated for the purposes of this petition.

OPPTS GLN 860.1500: Crop Field Trials

6a. Thirteen trials on apples provided adequate geographic representation. Pyriproxyfen residues were 0.05-0.18 ppm in/on apples harvested ~45 days following three applications at 0.11 lb ai/A/application. The HAFT residue level was 0.016 ppm (average of 0.014 and 0.018 ppm). Eight tests on pears provided adequate geographic representation. Pyriproxyfen residues were 0.01-0.09 ppm in/on pears harvested ~45 days following three applications at 0.11 lb ai/A/application. Based on the residue values obtained from apple and pear samples harvested following treatment at the proposed maximum use rates, the proposed tolerance level of 0.2 ppm for residues of pyriproxyfen in/on pome fruits is appropriate.

6b. Eight trials on walnuts provided adequate geographic representation. Pyriproxyfen residues were <0.02 ppm in walnuts harvested ~21 days after three applications at 0.11 lb ai/A/application. The proposed tolerance level of 0.02 ppm for residues of pyriproxyfen in/on walnuts is appropriate.

OPPTS GLN: 860.1520: Processed Food/Feed

7. Pyriproxyfen residues concentrated 4.9x in wet apple pomace processed from treated apples. Given a highest average field trail (HAFT) residue of 0.16 ppm, the maximum residues in wet pomace would be 0.78 ppm. Therefore, the proposed tolerance of 0.8 ppm for pyriproxyfen in wet apple pomace is appropriate.

OPPTS GLN: 860.1480: Meat/Milk/Poultry/Eggs

8. The maximum theoretical dietary intake of pyriproxyfen by beef and dairy cattle, respectively, is 1.69 and 1.29 ppm, based on tolerances proposed for residues in wet apple pomace, cotton seed, cotton gin byproducts, almond hulls and citrus pulp. Cows were dosed with pyriproxyfen at 2x, 5x, and 18x (beef) or 2x, 7x, and 23x (dairy) the maximum theoretical exposure for 28 days. Residues were <0.01 ppm in milk, liver, kidney, and muscle at all doses. Residues in fat were 0.01-0.03 ppm at 5x and 0.05-0.07 ppm at the 18x dose. Typically, tolerances are required on all animal commodities having detectable residue levels at a 10x dosing rate or below. For the computed maximum theoretical dietary burden (MTDB) of 1.69 ppm in beef cattle, this would include the 3 and 9 ppm dosing levels. The only commodity having detectable pyriproxyfen residues at these levels was fat: 0.01 - 0.03 ppm. Since the MTDB calculation is based on a nutritionally unbalanced diet and includes contributions from some animal feed items that are used only regionally, HED will not require the establishment of pyriproxyfen tolerances in fat

at this time. However, should future new uses include additional animal feed items, tolerances on animal commodities will be needed.

Other Considerations

9. There are no CODEX, Canadian, or Mexican tolerances for pyriproxyfen residues in/on pome fruits or walnuts; thus, international harmonization is not an issue at this time. Pyriproxyfen is scheduled as a new compound for JMPR review (both toxicology and residue chemistry) in 1999.

10. Adequate product chemistry data for the 97% SUMILARV technical product (EPA Reg. No. 10308-11) have been submitted in conjunction with PP#6F04737 (DP Barcode D228556, J. Garbus, 06-MAY-1997). The manufacturing impurities are not expected to pose residue problems at the maximum proposed use rate.

RECOMMENDATIONS

Provided Section B is revised as specified in Conclusion 1, RAB1 concludes there are no residue chemistry data requirements that would preclude a conditional registration of the proposed permanent tolerances for pyriproxyfen in/on pome fruits and walnuts while agency validation of the analytical method for apples and walnuts is conducted. A human-health risk assessment will be prepared as a separate document.

DETAILED CONSIDERATIONS

OPPTS 830 Series GLNs: Product Properties

Adequate product chemistry data for the 97% SUMILARV technical product (EPA Reg. No. 10308-11) have been submitted in conjunction with PP#6F04737 (DP Barcode D228556, J. Garbus, 06-MAY-1997). The manufacturing impurities are not expected to pose residue problems at the maximum proposed use rate.

OPPTS GLN 860.1200: Proposed Uses

The petitioner provided a specimen label for an 0.86 lb ai/gal emulsifiable concentrate (EC) formulation (product name: KNACK™ Insect Growth Regulator) including the proposed uses on apple, pear, and walnuts. Pyriproxyfen is intended for use in integrated pest management (IPM) or integrated resistance management (IRM) programs. The proposed use patterns are described below.

The 0.86 lb ai/gal EC formulation is proposed for multiple broadcast applications to **apples** at 30.5-50.0 grams ai/A (0.067-0.11 lb ai/A). Treatment for codling moth is to be made just prior to egg hatch, with a second application 14-21 days later; treatment for San Jose scale should be made at delayed dormant stage with oil or in cover sprays without oil; and treatment for spotted tentiform leafminer should be made when insects first appear, usually at prepink apple stage for first generation (an additional application may be made for the second generation). A 45-day PHI is established for apples.

The 0.86 lb ai/gal EC formulation is proposed for multiple broadcast applications to **pears** at 41.6-50.0 grams ai/A (0.092-0.110 lb ai/A). Treatment for codling moth is to be made just prior to egg hatch, with a second application 14-21 days later. Treatment for pear psylla and San Jose scale should be made at delayed dormant stage with a Supreme oil in 100-250 gals/A or in cover sprays made when insects (scale crawlers) first appear; a second application may be made at petal fall for pear psylla. A 45-day PHI is established for pears.

The 0.86 lb ai/gal EC formulation is proposed for multiple broadcast applications to **walnuts** at 41.6-50.0 grams ai/A (0.092-0.11 lb ai/A). Treatment for codling moth is to be made just prior to egg hatch, with a second application 14-21 days later; treatment for late season codling moth should be made at egg deposition with a second application 14-21 days later if necessary. A 21-day PHI is established for walnuts.

Applications are to be made in 100-400 gals/A; however, the application equipment (ground or aerial) was not specified on the label. A maximum rate of 100 grams ai/A (0.22 lb ai/A) is specified between petal fall and harvest, and a maximum seasonal application rate of 150 grams ai/A (0.33 lb ai/A) is established for apples, pears, and walnuts. A restricted entry interval of 12 hours is specified. The label specifies that applications are not to be made directly to water, to areas where surface water is present, to intertidal areas below the mean high water mark, or through any type of irrigation system.

Conclusions: The proposed use directions for the 0.86 lb ai/gal emulsifiable concentrate formulation on apples, pears, and walnuts are inadequate. Information concerning application equipment (i.e., ground or aerial equipment and amount of spray volume per acre) should be included on the proposed label. A revised Section B should be submitted.

OPPTS GLN 860.1300: Nature of the Residue - Plants

Apples

Valent has submitted data from a study (citation listed below) investigating the metabolism of [^{14}C]pyriproxyfen in apples. The in-life and analytical phases of the study were conducted by Ricerca, Inc., Environmental and Metabolic Fate (Painesville, OH).

MRID 44329506 Panthani, A.; Walsh, K. (1996) A Plant Metabolism Study with (carbon-14)-S-71639 (Pyriproxyfen) in Apple Trees: Lab Project Number: 95-0014: 6317-95-0014-EF-001: REPORT/S-71639. Unpublished study prepared by Ricerca, Inc. 210 p. {OPPTS860.1300}.

The petitioner conducted studies with [^{14}C]pyriproxyfen separately labeled in the pyridyl ring (labeled at the 2- and 6- positions) and the phenoxyphenyl ring (uniformly labeled). The pyridyl- and phenyl-labeled test substances were diluted with unlabeled emulsifiable concentrate formulation blank in water to form aqueous suspensions with specific activities of 781,440 dpm/μg and 743,700 dpm/μg (radiochemical purities of 98.2-100% and 99.2-100%). Three applications of each formulated test substance were made to two apple trees (approximately 4 years old) at a nominal rate of 60 grams ai/A/application (0.132 lbs ai/A/application). The total application rate was 180 grams ai/A (0.396 lbs ai/A; 1.2x the maximum proposed seasonal rate). Apple trees were sprayed shortly after petal fall (~4 months before harvest), and again at 60 and 45 days before harvest. Trees were enclosed in cages with the east and top sides covered with plastic for treatment. The plastic was removed from the east side approximately 15 minutes following treatment, and from the top within 1-3 days of treatment. All applications were made using a plastic hand-trigger sprayer.

Samples of mature apples and foliage were collected 45 days after the last (third) treatment (DAT). Samples were stored at -20°C until analysis. Five apples were collected from each treatment group for processing. Processing was initiated on the day of harvest at Ricerca. Initially apples were washed with acetonitrile, and the surface washes collected and pooled by treatment group. The washed apples were then slurried in a food processor. The slurried apples were centrifuged to separate the juice from the pomace. The juice was filtered and the pomace ground with dry ice. Juice and pomace samples were stored at -20°C until analysis.

Total radioactive residues (TRR)

Subsamples of apple foliage and pomace were homogenized with dry ice and total radioactive residues were determined in triplicate aliquots by liquid scintillation counting (LSC) following combustion. TRR in subsamples of apple surface wash and juice were determined by direct LSC. The TRR are presented in Table 1; the petitioner concluded that data from apple foliage would not aid in the identification of metabolites from the fruit and did not report leaf sample analyses. Detection limits (LODs) of 0.0001 ppm for apple juice and 0.0013 ppm for apple pomace were reported for direct LSC and combustion/LSC determinations. The petitioner calculated TRR in whole apples by summing the radioactivity in the surface wash, juice, and pomace fractions. In addition, TRR in pomace was determined in extracted pomace. The petitioner used the summed TRR values reported in parentheses (including TRR of extracted pomace) for all calculations of percent of TRR.

Table 1. Total radioactive residues (TRR) in samples of apples treated with [^{14}C]pyriproxyfen at 180 grams ai/A (0.396 lb ai/A; 1.2x the maximum seasonal rate).

Commodity	TRR, ppm [^{14}C]pyriproxyfen equivalents [a]	
	Pyridyl Label	Phenyl Label
Surface wash	0.002, 0.004	0.005, 0.004
Pomace	0.109, 0.142	0.187, 0.196
Juice	0.017, 0.023	0.015, 0.012
Apple [b]	0.119, 0.171 (0.185)	0.199 (0.188), 0.201

[a] TRR was calculated by the study reviewer from the μg value determined based on 800 g fresh weight of apple, except for whole apple where the TRR was based on the actual fresh weight of apple.

[b] TRR for whole apple fruit was calculated by summing the radioactivity of surface wash, pomace, and juice. TRR values in parentheses include radioactivity of extracted pomace tissue.

Extraction and hydrolysis of residues

Apple juice, surface wash, pomace and extracted pomace fractions were analyzed by HPLC to quantitate and characterize radioactive residues. The surface wash fraction was concentrated by rotary evaporation for HPLC analysis.

Because the majority of radioactivity in apple fruit was concentrated in the pomace fraction, subsamples of pomace were further extracted. Pomace samples were extracted twice with methanol and vacuum filtered. The methanol extract was combined with 50% aqueous acetonitrile (ACN) containing 1.0 N HCl. The solution was shaken at 40°C for 16 hours (overnight), and vacuum filtered. The methanol phase was concentrated by rotary evaporation, and the aqueous acid fraction was evaporated to dryness and redissolved in ACN:water (50:50, v:v, 1% acetic acid) for HPLC analysis. The remaining solids were washed with ACN and dried in a desiccator.

The distribution of ^{14}C-activity in individual extracts of apple juice, surface wash, and pomace is presented in Table 2.

Characterization/identification of residues

Apple juice, surface washes, and extracts of pomace were analyzed by HPLC to characterize residues of [^{14}C]pyriproxyfen in apples. Analyses were conducted using the following HPLC systems: (i) Maxsil C18 column and gradient mobile phase of acidified water (1% acetic acid):ACN; (ii) Spherisorb C6 column and gradient mobile phase of acidified water (1% acetic acid):ACN; or (iii) Zorbax SB-phenyl column and gradient mobile phase of acidified water (1% acetic acid):methanol. Nonlabeled standards were detected by UV (254 nm), and radioactivity was quantitated by fraction collection and radioactive flow detection (LSC).

Metabolites were identified by cochromatography with the following reference standards: pyriproxyfen; 4-(4-hydroxyphenoxy)phenyl (RS)-2-(2-pyridyloxy)propyl ether (4'-OH-PYR); (RS)-5-hydroxy-2-[1-methyl-2-(4-phenoxyphenoxy)ethoxy]pyridine (5"-OH-PYR); 4-hydroxyphenyl (RS)-2-(2-pyridyloxy)propyl ether (DPH-PYR); (RS)-2-hydroxypropyl 4-phenoxyphenyl ether (POPA); 4-(4-hydroxyphenoxy)phenyl (RS)-2-hydroxypropyl ether (4'-OH-POPA); 4-phenoxyphenol (POP); 4,4'-oxydiphenol (4'-OH-POP); (RS)-2-(2-pyridyloxy)propyl alcohol (PYPA); (RS)-2-(2-pyrdyloxy)propionic acid (PYPAC); 2-hydroxypyridine (2-OH-PY); 1-[2-methyl-2-(2-pyridyloxy)ethyl]-β-D-glucopyranose (PYPA-Glc); 4-hydroxyphenyl (RS)-2-hydroxypropyl ether (DPH-POPA); and methyl (RS)-2-(2-pyridyloxy)propionate (PYPAC-ME) (see Figure 1 for structures of identified metabolites).

Isolated metabolites were analyzed by TLC on pre-coated silica gel plates (60 LK6DF) using a solvent system of toluene:ethyl acetate:acetic acid (7:3:0.1, v:v:v). Standards (^{14}C-labeled and nonlabeled) of the expected metabolites were co-chromatographed with the sample. Radioactivity was quantitated with a Bioscan Imaging Scanner System 200, and nonlabeled standards were observed under UV light.

Major radioactivity observed in apple juice and the methanol fraction of extracted pomace was subjected to further isolation and purification for characterization of metabolites. The major metabolite in apple juice samples was purified by open column chromatography (glass column packed with C18 silica slurry in methanol). Residues were eluted with water and methanol:water (60:40, v:v). Fractions containing radioactivity were pooled, concentrated, and purified by repeated HPLC collections.

Conjugated metabolites in apple juice and pomace were subjected to acid hydrolysis (6.0 N HCl at 70 or 80°C for 2 hours). The hydrolyzed fractions were separated into individual components by various HPLC systems. Metabolite identification of the isolated fraction (free metabolite) was then determined by GC/MS or LC/MS analysis.

The nonextractable residues of apple pomace were sequentially subjected to mild acid hydrolysis (1.0 N HCl at 40°C for 16 hours), strong acid hydrolysis (6.0 N HCl at 80-85°C for 2 hours), and base hydrolysis (1.0 N NaOH at 40°C for 16 hours). The base hydrolysates, which had the highest radioactivity, were reserved for HPLC analysis.

A summary of the characterized and identified ^{14}C-residues in/on apple juice, surface wash, and pomace is presented in Table 3.

Table 2. Distribution and characterization/identification of radioactive residues in/on apples treated with [^{14}C]pyriproxyfen at 180 grams ai/A (0.396 lb ai/A; 1.2x the proposed maximum seasonal rate).

Fraction	% TRR	ppm [a]	Characterization/Identification [b]		
Phenyl Label					
Whole Apple (TRR = 0.188 ppm)					
ACN Surface wash	2.7	0.005	HPLC analysis resolved:		
			Pyriproxyfen	2.4% TRR	0.004 ppm
			4'-OH-PYR (free)	0.1% TRR	<0.001 ppm
			Diffuse	0.1% TRR	<0.001 ppm
Juice	7.4	0.014	HPLC analysis resolved:		
			Pyriproxyfen	<0.1% TRR	<0.001 ppm
			4'-OH-PYR (free)	<0.1% TRR	<0.001 ppm
			4'-OH-POPA(conj)	0.9% TRR	0.002 ppm
			4'-OH-POP (conj)	0.4% TRR	0.001 ppm
			5"-OH-PYR (conj)	0.1% TRR	<0.001 ppm
			DPH-PYR (conj)	0.6% TRR	0.001 ppm
			POP (free)	0.2% TRR	<0.001 ppm
			POPA (conj)	0.2% TRR	<0.001 ppm
			Polar unknowns	2.4% TRR	0.004 ppm
			Unknown 21.87 Rt	0.4% TRR	0.001 ppm
			Unknown 28.93 Rt	0.5% TRR	0.001 ppm
			Diffuse	1.8% TRR	0.003 ppm
Pomace	90.4	0.170 [c]	Sequentially extracted twice with methanol, and ACN:water (50:50, v:v, 1.0 N HCl)		
Methanol	70.7	0.133	HPLC analysis resolved:		
			Pyriproxyfen	48.8% TRR	0.092 ppm
			4'-OH-PYR (free)	10.8% TRR	0.020 ppm
			4'-OH-POPA (conj)	1.1% TRR	0.002 ppm
			5"-OH-PYR(conj)	0.6% TRR	0.001 ppm
			DPH-PYR (conj)	1.1% TRR	0.002 ppm
			DPH-PYR (free)	0.5% TRR	0.001 ppm
			POP (conj)	0.6% TRR	0.001 ppm
			POP (free)	0.5% TRR	0.001 ppm
			POPA (conj)	0.9% TRR	0.002 ppm
			POPA (free)	0.5% TRR	0.001 ppm
			Polar unknowns	0.7% TRR	0.001 ppm
			Unknown 28.93 Rt	1.0% TRR	0.002 ppm
			Diffuse	3.6% TRR	0.007 ppm
ACN:Water	4.3	0.008	HPLC analysis resolved:		
			Pyriproxyfen	0.6% TRR	0.001 ppm
			4'-OH-PYR (free)	0.3% TRR	0.001 ppm
			DPH-PYR (free)	0.9% TRR	0.002 ppm
			POPA (free)	0.4% TRR	0.001 ppm
			Polar unknowns	1.0% TRR	0.002 ppm
			Diffuse	1.1% TRR	0.002 ppm
Nonextractable	14.9	0.028	Sequentially subjected to mild acid (1.0 N HCl), strong acid (6.0 N HCl), and base (1.0 N NaOH) hydrolysis.		

(continued; footnotes follow)

Table 2 (*continued*).

Fraction	% TRR	ppm [a]	Characterization/Identification [b]		
Mild acid hydrolysate	0.4	0.001	Not further analyzed (N/A.)		
Strong acid hydrolysate	0.5	0.001	N/A.		
Base hydrolysate	10.4	0.019	HPLC analysis resolved:		
			DPH-PYR (conj)	3.1% TRR	0.006 ppm
			4'-OH-POPA	0.9% TRR	0.002 ppm
			POPA	0.8% TRR	0.002 ppm
			Polar unknowns	1.9% TRR	0.004 ppm
			Unknown ~22 Rt	1.7% TRR	0.003 ppm
			Diffuse	2.0% TRR	0.002 ppm
Solids	3.7	0.007	N/A.		
Pyridyl Label					
Whole Apple (TRR = 0.185 ppm)					
ACN Surface wash	2.2	0.004	HPLC analysis resolved:		
			Pyriproxyfen	2.0% TRR	0.004 ppm
			4'-OH-PYR (free)	0.1% TRR	<0.001 ppm
			Diffuse	0.1% TRR	<0.001 ppm
Juice	13.0	0.024	HPLC analysis resolved:		
			Pyriproxyfen	<0.1% TRR	<0.001 ppm
			4'-OH-PYR (free)	<0.1% TRR	<0.001 ppm
			PYPA (conj)	1.7% TRR	0.003 ppm
			PYPA (free)	2.5% TRR	0.004 ppm
			PYPAC (free)	0.7% TRR	0.001 ppm
			DPH-PYR (conj)	0.2% TRR	<0.001 ppm
			Polar unknowns	4.6% TRR	0.009 ppm
			Unknown 21.07 Rt	1.7% TRR	0.003 ppm
			Unknown 24.73 Rt	0.4% TRR	0.001 ppm
			Diffuse	1.2% TRR	0.002 ppm
Pomace	83.7	0.155 [c]	Sequentially extracted twice with methanol, and ACN:water (50:50, v:v, 1.0 N HCl)		
Methanol	69.2	0.128	HPLC analysis resolved:		
			Pyriproxyfen	51.9% TRR	0.096 ppm
			4'-OH-PYR (free)	8.8% TRR	0.016 ppm
			PYPA (conj)	0.9% TRR	0.002 ppm
			PYPA (free)	1.2% TRR	0.002 ppm
			PYPAC (free)	0.8% TRR	0.002 ppm
			DPH-PYR (conj)	0.8% TRR	0.001 ppm
			DPH-PYR (free)	0.3% TRR	0.001 ppm
			5"-OH-PYR (conj)	0.2% TRR	<0.001 ppm
			Polar unknowns	1.0% TRR	0.002 ppm
			Unknown 21.07 Rt	0.9% TRR	0.002 ppm
			Diffuse	2.5% TRR	0.005 ppm

(continued; footnotes follow)

Table 2 (*continued*).

Fraction	% TRR	ppm [a]	Characterization/Identification [b]		
ACN:Water	3.2	0.006	HPLC analysis resolved:		
			Pyriproxyfen	0.5% TRR	0.001 ppm
			4'-OH-PYR (free)	0.3% TRR	0.001 ppm
			PYPA (free)	0.3% TRR	0.001 ppm
			DPH-PYR (free)	0.7% TRR	0.001 ppm
			Polar unknowns	0.9% TRR	0.002 ppm
			Diffuse	0.6% TRR	<0.001 ppm
Nonextractable	12.4	0.023	Sequentially subjected to mild acid (1.0 N HCl), strong acid (6.0 N HCl), and base (1.0 N NaOH) hydrolysis.		
Mild acid hydrolysate	0.5	0.001	N/A.		
Strong acid hydrolysate	0.5	0.001	N/A.		
Base hydrolysate	7.8	0.014	HPLC analysis resolved:		
			DPH-PYR	1.8% TRR	0.003 ppm
			PYPA	1.1% TRR	0.002 ppm
			Polar unknowns	0.8% TRR	0.001 ppm
			Unknown ~28 Rt	1.3% TRR	0.002 ppm
			Diffuse	2.8% TRR	0.006 ppm
Solids	3.7	0.007	N/A.		

[a] Percent of TRR and ppm values were reported by the petitioner for samples used for HPLC analysis, except for pomace.

[b] Conjugated metabolites were identified as the free metabolite following acid hydrolysis.

[c] Ppm value for pomace was calculated by the study reviewer from the %TRR in the nonextracted pomace fraction.

Table 3. Summary of radioactive residues characterized/identified in apples treated with [^{14}C]pyriproxyfen at 180 grams ai/A (0.396 lb ai/A; 1.2x the proposed maximum seasonal rate).

Fraction	Phenyl Label (TRR = 0.188 ppm)		Pyridyl Label (TRR = 0.185 ppm)	
	% TRR	ppm	% TRR	ppm
Identified [a]				
Pyriproxyfen	51.8	0.097	54.4	0.101
4'-OH-PYR (free)	11.3	0.021	9.2	0.017
4'-OH-POPA (conj)	2.9	0.006	--	--
4'-OH-POP (conj)	0.4	0.001	--	--
5"-OH-PYR (conj)	0.7	0.001	0.2	<0.001
DPH-PYR (conj)	4.8	0.009	2.8	0.004
DPH-PYR (free)	1.4	0.003	1.0	0.002
POP (conj)	0.8	0.001	--	--
POP (free)	0.5	0.001	--	--
POPA (conj)	1.9	0.004	--	--
POPA (free)	0.9	0.002	--	--
PYPA (conj)	--	--	3.7	0.007
PYPA (free)	--	--	4.0	0.007
PYPAC (free)	--	--	1.5	0.003
Total identified	**77.4**	**0.146**	**76.7**	**0.142**
Characterized				
Polar Unknowns	6.0	0.011	7.2	0.014
Unknown 21.07 Rt	--	--	2.5	0.005
Unknown 21.87 Rt	0.4	0.001	--	--
Unknown 22 Rt	1.7	0.003	--	--
Unknown 24.73 Rt	--	--	0.4	0.001
Unknown 28 Rt	--	--	1.3	0.002
Unknown 28.93 Rt	1.5	0.003	--	--
Diffuse	8.6	0.014	7.2	0.014
Mild acid hydrolysate	0.4	0.001	0.5	0.001
Strong acid hydrolysate	0.5	0.001	0.5	0.001
Total identified/characterized	**96.5**	**0.180**	**96.3**	**0.180**
Nonextractable	3.7	0.007	3.7	0.007

[a] See Figure 1 for the full chemical name and chemical structure of the identified metabolites.

Storage stability

Samples of apple commodities were stored frozen (~-20°C) prior to analysis. Although analysis dates were not submitted, based on the latest dated chromatogram (TLC), samples

were stored for at least 179 days (~6 months) between harvest and final analysis. An aliquot of pomace stored frozen during the duration of the study was extracted and analyzed by HPLC to verify the stability of pyriproxyfen metabolites under freezer conditions. An aliquot of apple juice was also reanalyzed by HPLC at completion of the analytical phase of the study. The petitioner indicated that metabolic profiles of apple juice and pomace were virtually identical in the initial and final analyses, indicating that pyriproxyfen metabolites are stable in apple matrices under freezer conditions. No additional storage stability data are required to support this study.

Conclusions

The apple metabolism study is adequate. Total radioactive residues were 0.002-0.004 ppm in surface wash, 0.109-0.142 ppm in pomace, 0.017-0.023 ppm in juice, and 0.119-0.185 ppm in whole apples (summed total of washings, juice, and pomace) treated with pyridyl labeled [^{14}C]pyriproxyfen, and 0.004-0.005 ppm in surface wash, 0.187-0.196 ppm in pomace, 0.012-0.015 ppm in juice, and 0.188-0.201 ppm in whole apples (summed total of washings, juice, and pomace) treated with phenyl labeled [^{14}C]pyriproxyfen at 1.2x the maximum seasonal rate and harvested 45 days following the last of three applications.

The nature of the residue in apple is understood. The major metabolite identified in the apple surface washing, juice, and pomace from apples treated with both phenyl- and pyridyl-labeled pyriproxyfen was the parent, pyriproxyfen, present at 51.8% (0.097 ppm) and 54.4% (0.101 ppm) of TRR, respectively. The second most prevalent metabolite identified in apples treated with both phenyl- and pyridyl-labeled pyriproxyfen was the metabolite 4'-OH-PYR, present at 11.3% (0.021 ppm) and 9.2% (0.017 ppm) of TRR, respectively. Other metabolites identified in apples treated with both phenyl- and pyridyl-labeled pyriproxyfen were: 5"-OH-PYR (conjugated), and DPH-PYR (free and conjugated). In addition, the following metabolites were identified in apples treated with phenyl-labeled pyriproxyfen: 4'-OH-POPA (conjugated), 4'-OH-POP (conjugated), POP (free and conjugated), and POPA (free and conjugated) ranging 0.4-2.9% of TRR (0.001-0.006 ppm). In addition, the following metabolites were identified in apples treated with pyridyl-labeled pyriproxyfen: PYPA (free and conjugated), and PYPAC (free) ranging 1.5-5.0% of TRR (0.003-0.009 ppm). Conjugated metabolites were identified as the free metabolite following acid hydrolysis.

Based on the submitted apple metabolism study, the major metabolic pathway in apples is by hydroxylation and cleavage of the phenoxy ether linkage. Primary metabolites formed are further metabolized to more polar products by oxidation or conjugation reactions. Similar metabolic pathways were observed for the metabolism of pyriproxyfen in cotton, goats, and hens.

Accordingly, the Metabolism Assessment Review Committee (MARC) decided on 10-NOV-1998 that the residue of concern in plants is pyriproxyfen per se (D250953, W. Donovan & W.

Dykstra, 19-NOV-1998), unless a significantly different metabolic pathway is identified for a given crop. This finding confirms the 15-JUL-1996 MARC decision rendered for cotton.

Figure 1. Pyriproxyfen and its metabolites in apples.

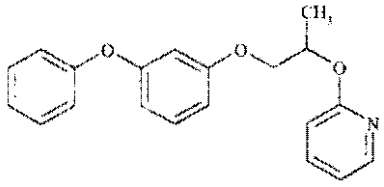
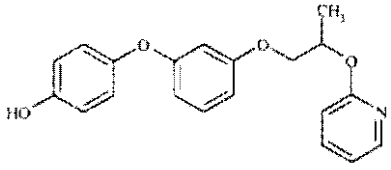
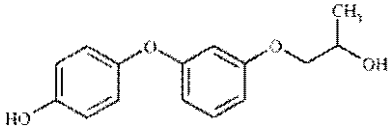
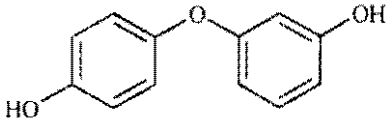
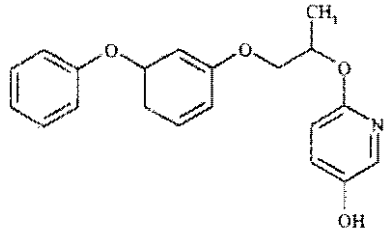
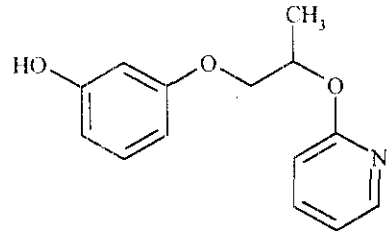
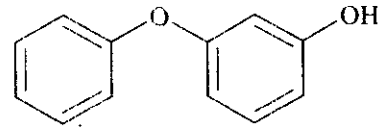
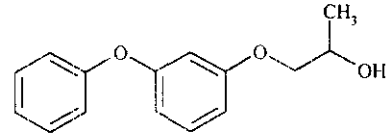
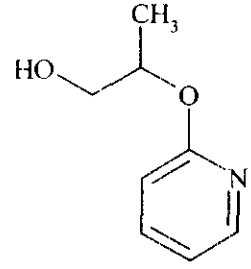
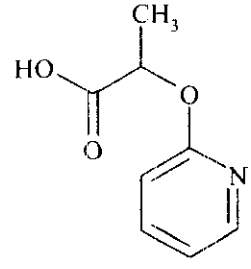
Common Name Chemical Name	Structure	Substrate
Pyriproxyfen 4-phenoxyphenyl (*RS*)-2-(2-pyridyloxy)propyl ether		Phenyl- and pyridyl-labeled apple
4'-OH-PYR 4-(4-hydroxyphenoxy)phenyl (*RS*)-2-(2-pyridyloxy)propyl ether		Phenyl- and pyridyl-labeled apple
4'-OH-POPA 4-(4-hydroxyphenoxy)phenyl (*RS*)-hydroxypropyl ether		Phenyl-labeled apple
4'-OH-POP 4,4-oxydiphenol		Phenyl-labeled apple
5"-OH-PYR (*RS*)-5-hydroxy-2-[1-methyl-2-(4-phenoxyphenoxy)ethoxyl]pyridine		Phenyl- and pyridyl-labeled apple

Figure 1 (*continued*).

Common Name Chemical Name	Structure	Substrate
DPH-PYR 4-hydroxyphenyl (*RS*)-2-(2-pyridyloxy)propyl ether		Phenyl- and pyridyl-labeled apple
POP 4-phenoxyphenol		Phenyl-labeled apple
POPA (*RS*)-2-hydroxypropyl 4-phenoxyphenyl ether		Phenyl-labeled apple
PYPA (*RS*)-2-(2-pyridyloxy)propyl alcohol		Pyridyl-labeled apple
PYPAC (*RS*)-2-(2-pyridyloxy)propionic acid		Pyridyl-labeled apple

OPPTS GLN 860.1300: Nature of the Residue - Animals

No animal metabolism data were submitted with this petition. Ruminant and poultry metabolism studies have previously been submitted and reviewed (PP#6F04737, DP Barcode D228556, J. Garbus, 06-MAY-1997) in conjunction with a petition for cotton. The animal metabolism studies demonstrated that the transfer of ^{14}C residues to tissues was low. Total residues in goat milk, muscle, and tissues accounted for less than 2% of the TRR. Total residues in poultry eggs, muscle, and tissue accounted for ~2.7% of the TRR.

OPPTS GLN 860.1340: Residue Analytical Methods

Residue Analytical Methods - Plant Commodities

Data collection methods: Samples of apples and pears (1994 and 1995 trials) were initially analyzed for residues of pyriproxyfen and its degradates DPH-PYR, POPA, 4'-OH-PYR, and 5"-OH-PYR using GC/NPD method RM-33P-1 and HPLC method RM-33M-1, respectively. Once pyriproxyfen and 4'-OH-PYR were determined to be the residues of concern in the apple metabolism study, GC/NPD and HPLC method RM-33P-1-3 entitled "Determination of Pyriproxyfen and 4'-OH-Pyriproxyfen Residues in Apples, Pears, and Citrus Fruits" was used to analyze the apple and pear samples from field trials conducted in 1996. Method RM-33P-1-3 was previously reviewed in conjunction with a petition for a tolerance for residues in/on cotton (PP#6F04737, DP Barcode D228556, J. Garbus, 06-MAY-1997).

Samples of walnuts from the submitted field trial were analyzed for residues of pyriproxyfen and its metabolite 4'-OH-PYR using GC/NPD and HPLC method RM-33N-2, entitled "Determination of Pyriproxyfen and 4'-OH-Pyriproxyfen in Nutmeats". This method is similar to Method RM-33P-1-3; minor modifications were made to the method for the analysis of walnut commodities.

Brief descriptions of the methods follow; sample calculations and chromatograms were included in the submission.

Method RM-33P-1: Residues of pyriproxyfen were extracted from apple and pear fruit by blending with acetone and filtration. The acetone extraction procedure was repeated, and extracts combined. Residues were then partitioned with dichloromethane (DCM):5% aqueous solution of sodium chloride. The DCM phase was filtered through sodium sulfate, and the aqueous phase was partitioned again with DCM. The DCM phases were combined and evaporated just to dryness using rotary evaporation in a water bath at $<40°C$. Residues were redissolved in hexane and purified further by silica gel chromatography. Pyriproxyfen residues were eluted with hexane:diethyl ether (15:1, v:v) and evaporated just to dryness. Pyriproxyfen residues were redissolved in toluene for GC analysis using a nitrogen-

phosphorous specific flame-ionization detector (NPD). The limit of detection (LOD) was reported as 0.01 ppm. The limit of quantitation (LOQ) was 0.02 ppm.

Method RM-33M-1: Residues of DPH-PYR, POPA, 4'-OH-PYR, and 5"-OH-PYR were extracted from apple and pear fruit by blending with methanol:water (4:1, v:v) and filtration. The methanol:water extraction procedure was repeated, and extracts combined. Methanol was removed from the combined extracts using rotary evaporation in a water bath at $<40°C$. The pH of the remaining aqueous residue was adjusted to pH 0.9 by adding 6 N HCl, and heated to reflux for 2 hours. After cooling overnight, the pH of the hydrolyzed sample was adjusted to pH 7 by adding 50% and 10% aqueous sodium hydroxide. Residues were then partitioned with acetone washed sodium chloride and ethyl acetate. The ethyl acetate phase was collected through a filter funnel with sodium sulfate, and the aqueous phase partitioned twice again with ethyl acetate. The ethyl acetate phases were combined, 1% BHT in methanol was added, and the ethyl acetate evaporated just to dryness using rotary evaporation in a water bath at $<40°C$. Residues were redissolved in acetone and purified further by silica gel chromatography. Residues were eluted with hexane:acetone (70:30, v:v), BHT (1%) in methanol was added to the eluate, and the solvent evaporated just to dryness. Residues were redissolved in acetone. Residues of DPH-PYR were analyzed by GC/NPD. Residues of POPA, 4'-OH-PYR, and 5"-OH-PYR were solvent exchanged with acetone, evaporated to dryness, redissolved in methanol:water (3:1, v:v), and filtered for HPLC analysis using a C18 column, gradient mobile phase of methanol:tetrahydrofuran (2:3, v:v) and acidified water (0.04% phosphoric acid, v:v), and fluorescence (FLD) detection. The reported LOD was 0.05 ppm for each analyte. The LOQ was 0.10 ppm for each analyte.

Method RM-33P-1-3: This method is based on Method RM-33P-1 with the addition of preparation steps for 4'-OH-PYR. Residues of pyriproxyfen and 4'-OH-PYR were extracted from apple fruit, processed apples (juice and pomace), and pear fruit by blending with acetone and filtration. The acetone extraction procedure was repeated twice, combining the extracts. The acetone was evaporated by rotary evaporation in a water bath at $<40°C$, and ethyl acetate added to obtain an aqueous residue. Residues were then partitioned with acetonitrile (ACN) and hexane. The ACN phase was collected and the hexane phase partitioned twice again with ACN. The ACN phases were combined and rotary evaporated just to dryness. Residues were then partitioned with DCM:5% aqueous solution of sodium chloride. The DCM phase was filtered through sodium sulfate, and the aqueous phase partitioned twice again with DCM. The DCM phases were combined and evaporated just to dryness using rotary evaporation in a water bath at $<40°C$. Residues were redissolved in ethyl acetate and the solvent rotary evaporated. Residues were redissolved in hexane:ethyl acetate (for pyriproxyfen 80:20, v:v; for 4'-OH-PYR 60:40, v:v) and purified further by silica gel chromatography. Pyriproxyfen residues were eluted with hexane:ethyl acetate (80:20, v:v) and evaporated just to dryness. Pyriproxyfen residues were redissolved in toluene for GC/NPD analysis. 4'-OH-PYR residues were eluted with hexane:ethyl acetate (60:40, v:v) and evaporated just to dryness. 4'-OH-PYR residues were redissolved in methanol:water (4:1, v:v) and filtered for HPLC/FLD analysis using an ODS(3) column, and gradient mobile phase of methanol:tetrahydrofuran (2:3, v:v)

and acidified water (0.05% phosphoric acid, v:v). The LOD for pyriproxyfen and 4'-OH-PYR was reported as 0.01 ppm for each analyte. The limit of quantitation (LOQ) for both analytes was reported as 0.02 ppm.

Method RM-33N-2: Residues of pyriproxyfen and 4'-OH-PYR were extracted from walnut nutmeats with acetone, sequentially partitioned with DCM:5% aqueous solution of sodium chloride and hexane:ACN, and purified by silica gel chromatography as described above for Method RM-33P-1-3. Pyriproxyfen residues were eluted with hexane:diethyl ether (15:1, v:v) followed by hexane:acetone (7:3, v:v), and 4'-OH-PYR residues were eluted with additional hexane:acetone (7:3, v:v). The eluates were evaporated just to dryness. An additional hexane:ACN partitioning step was added prior to analysis to remove the last traces of oil and improve chromatography. Hexane (saturated with ACN) was added to the pyriproxyfen dried eluate, followed by the addition of ACN (saturated with hexane). Following sonication and vortexing, the ACN phase was collected and the hexane phase partitioned twice again with ACN (saturated with hexane). The combined ACN fraction was evaporated just to dryness, and redissolved in toluene for GC/NPD analysis. As for pyriproxyfen, 4'-OH-PYR residues were initially partitioned with hexane and ACN; however, the hexane phase was discarded, and the ACN phase was partitioned twice again with hexane (saturated with ACN). The combined ACN fraction was evaporated just to dryness, redissolved in methanol:water (4:1, v:v), and filtered for HPLC analysis using an ODS(3) column, gradient mobile phase of methanol:tetrahydrofuran (2:3, v:v) and acidified water (0.04% phosphoric acid, v:v), and either FLD or variable wavelength (VWD) detection. The LOD for pyriproxyfen and 4'-OH-PYR in nutmeats was reported as 0.01 ppm for each analyte. The validated LOQ for both analytes is 0.02 ppm in walnuts.

The petitioner submitted method validation data conducted on apples and walnuts prior to analysis of the field trial samples. Untreated apples were fortified with pyriproxyfen and 4'-OH-PYR each at 0.02-0.50 ppm and analyzed using GC and HPLC methods RM-33P-1, RM-33M-1, and RM-33P-1-3. Untreated walnuts were fortified with pyriproxyfen and 4'-OH-PYR each at 0.02-0.1 ppm and analyzed using GC and HPLC method RM-33N-2 without the additional hexane:ACN partitioning step. In addition, untreated apple, apple juice, apple pomace, pear, and walnut samples were fortified with pyriproxyfen and 4'-OH-PYR each at 0.01-0.50 ppm and analyzed concurrently with the treated field samples. The results of the method validation study and concurrent method validation study are presented in Table 4.

Table 4. Method validation and concurrent method recoveries of pyriproxyfen residues from fortified samples of commodities from the field trial and processing studies using GC/NPD and HPLC methods.

Commodity	Fortification Level (ppm)	% Recovery [a]	
		Pyriproxyfen	4'-OH-PYR
Method Validation			
Apples	0.02-0.50	91-113 (9) [b] 95-103 (3)	68 (1); 70-98 (9) [c] 83-88 (3)
Walnuts	0.02-0.10	81-94 (9)	86-94 (9)
Concurrent Method Recovery			
Apples	0.020-0.500	80-107 (18)	65, 67 (2); 75-83 (6) [c] 76-104 (12)
Apple, juice	0.020-0.01	91, 97 (2)	100, 107 (2)
Apple, pomace	0.04-0.20	89, 90 (2)	92, 96 (2)
Pears	0.010-0.200	85-119 (13)	78-98 (4) [c] 82-110 (8)
Walnuts	0.01-0.10	77-103 (12)	83-113 (14) [d]

[a] Analyses were performed using methods RM-33P-1-3 (apples, apple juice and pomace, and pears) and RM-33N-2 (walnuts) unless otherwise noted. The number of recovery analyses are reported in parentheses.

[b] Using Method RM-33P-1

[c] Using Method RM-33M-1.

[d] Two samples were analyzed with the alternate fluorescence detector; all other results were obtained using variable wavelength detection.

Independent laboratory validation - GC/NPD and HPLC Method RM-33P-1-3: The petitioner submitted the following data pertaining to the independent laboratory validation of GC/NPD and HPLC Method RM-33P-1-3 for determining residues of pyriproxyfen and 4'-OH-PYR in/on apples and oranges.

MRID 44329507 Wood, B. (1997) Independent Laboratory Validation of Valent Analytical Method RM-33P-1-3 for Determining Pyriproxyfen and 4'-OH-Pyriproxyfen Residues in/on Apples and Oranges: Lab Project Number: 120.001: VP-11866: 120.01. Unpublished study prepared by North Coast Labs., Ltd. 100 p. {OPPTS 860.1340}

The validation study was conducted by North Coast Laboratories, Ltd. (Arcata, CA) using untreated samples of apples and oranges from the field trials. HED notes that data concerning oranges are not pertinent to the petition on pome fruits and walnuts and are not presented here. Apple samples were fortified with pyriproxyfen and 4'-OH-PYR each at 0.02 ppm, representing the LOQ, and at higher levels of 0.20 ppm (pyriproxyfen) and 0.04 ppm (4'-OH-PYR). The results of the independent validation study are presented in Table 5. The only modification to the method made by the test laboratory was to rinse the silica gel column with

a strong solvent (hexane:ethyl acetate, 20:80, v:v) followed by a rinse with the application solvent (hexane:ethyl acetate, 80:20, v:v) to remove an interference peak. Sample calculations and representative chromatograms were included in the submission. The test laboratory stated that analysis of a set of 7 samples for both pyriproxyfen and 4'-OH-PYR required 20 person-hours or 2.5 calendar days. The test laboratory noted that with experience the time required could most likely be reduced to 10-12 hours (1.25-1.5 calendar days) for a set of 7 samples.

Table 5. Independent laboratory validation of GC/NPD and HPLC Method RM-33P-1-3 using apple samples.

Commodity	Fortification Level, ppm	Recovery, %	
		Pyriproxyfen	4'-OH-PYR
Apple	0.02	79, 80	80, 100
	0.04	--	71, 85
	0.20	78, 82	--

Radiovalidation of the proposed enforcement method

Valent has submitted radiovalidation data (citation listed below) for a GC/NPD and HPLC method (RM-33P-1-3) for determination of pyriproxyfen and unconjugated 4'-OH-PYR in apple pomace. Radiovalidation was conducted by Valent Technical Center (Dublin, CA).

MRID 44329508 Pensyl, J. (1997) Radiovalidation of the Residue Analytical Method for Determining Residues of Pyriproxyfen and Its Degradates in Apples: Lab Project Number: VP-11590: VL-005-04: RM-33P-1-3. Unpublished study prepared by Valent U.S.A. Corp.100 p. {OPPTS 860.1340}.

Samples of apple pomace from the [^{14}C]pyriproxyfen apple metabolism study were utilized. Juice samples were not analyzed for radiovalidation because pyriproxyfen and 4'-OH-PYR were not detected (<0.01 ppm) in the juice fraction from the apple metabolism study. The TRR determined for apple pomace by combustion/LSC prior to extraction and residue analysis were converted from a whole apple fruit basis to a pomace basis. The residues determined by the residue method were converted to pyriproxyfen equivalents and corrected for concurrent method recoveries. Comparison of residue levels determined by the radiochemical and residue methods are presented in Table 6.

Duplicate samples of apple pomace were extracted with acetone, partitioned with DCM and water, and cleaned-up with silica gel column chromatography (as described above). Pyriproxyfen residues were quantitated by GC/NPD and residues of 4'-OH-PYR were analyzed by HPLC. The reported LOD and LOQ are 0.01 and 0.02 ppm, respectively, in all apple matrices. Matrix interferences were observed in the untreated apple pomace. The

interfering peak was resolved by re-analysis for pyriproxyfen using an alternate GC column (DB-5) and for 4'-OH-PYR by slightly modifying the HPLC mobile phase gradient.

Table 6. Comparison of residues of pyriproxyfen and 4'-OH-PYR determined in samples of apple pomace from the [^{14}C]pyriproxyfen apple metabolism study and determined using the proposed GC/NPD and HPLC method (RM-33P-1-3) enforcement method.

Analytes	Residue Method				Radiometric Method Pyriproxyfen Equivalents (ppm)
	Residues (ppm)	Pyriproxyfen Equivalents (ppm) [a]	Concurrent Method % Recovery	Corrected Pyriproxyfen (ppm)	
Pyriproxyfen	0.299, 0.335	0.317	87	0.365	0.466 [b]
4'-OH-PYR	0.050, 0.061	0.053	78	0.068	0.078 [c]

[a] Mean of 4'-OH-PYR results were converted to pyriproxyfen equivalents by multiplying by the molecular weight of pyriproxyfen and dividing by the molecular weight of 4'-OH-PYR.
[b] Converted from whole apple fruit (0.096 ppm) to pomace basis.
[c] Converted from whole apple fruit (0.016 ppm) to pomace basis.

Conclusions

The GC/NPD and HPLC methods RM-33P-1-3 and RM-33N-2 are adequate for the purposes of residue data collection for pyriproxyfen and 4'-OH-PYR residues in/on apple, processed apple, pear, and walnut commodities. Adequate independent laboratory validation (on apples), method validation, and concurrent method recovery have been submitted for these methods. Method RM-33-P-1-3 has been adequately radiovalidated for pyriproxyfen and 4'-OH-PYR, using samples of apple pomace from the [^{14}C]pyriproxyfen apple metabolism study.

Method RM-33N-2 is fundamentally similar to RM-33P-1-3 which has also undergone independent laboratory validation (ILV), radiovalidation, and successful petition method validation in conjunction with a tolerance petition for use of pyriproxyfen on cotton (PP#6F04737. DP Barcode D228556, J. Garbus, 06-MAY-1997).

Agency validation of methods RM-33P-1-3 and RM-33N-2 for apples and walnuts, respectively, is needed before a conclusion can be reached about the suitability of these methods for tolerance enforcement.

Residue Analytical Method - Animal Commodities

Data collection methods: Samples of milk, cream, and animal tissues from the cattle feeding study were analyzed for residues of pyriproxyfen and its metabolites, 4'-OH-PYR, POP, and

2,5-OH-pyridine using a GC/NPD and HPLC Methods RM-33G-2 (pyriproxyfen, 4'-OH-PYR, and POP in milk), RM-33G-3 (2,5-OH-pyridine in milk), RM-33T-1 (pyriproxyfen in tissues), RM-33T-2 (4'-OH-PYR in tissues), RM-33T-3 (POP in liver and kidney), and RM-33T-4 (2,5-OH-pyridine in liver and kidney). In the cattle feeding study, sample analyses were performed by Valent Technical Center, Dublin, CA. The reported LOD and LOQ were 0.01 and 0.02 ppm, respectively, for each analyte in all matrices, except in a few cases where interferences in the controls increased the LOD and LOQ to 0.02 and 0.04 ppm, respectively. A brief description of the methods follow; sample calculations and chromatograms were submitted.

Method RM-33G-2: Residues of pyriproxyfen, 4'-OH-PYR, and POP (including conjugates) are extracted from milk with ethyl acetate:methanol (2:1, v:v). The organic solvents are evaporated by rotary evaporation, and residues partitioned into ethyl acetate. Ethyl acetate is removed by evaporation, and the milk fats removed by partitioning with hexane and ACN. The ACN phase is rotary evaporated and the residues redissolved in acetone. The resulting extract is split into two portions. One portion is rotary evaporated, residues are dissolved in hexane:ethyl acetate (10:1, v:v), and subjected to alumina column cleanup. The collected eluate is rotary evaporated, and residues redissolved in toluene for analysis of residues of pyriproxyfen by GC/NPD. The other portion is rotary evaporated and residues hydrolyzed with 1 N HCl (heated for 2 hours) to release conjugates of POP and 4'-OH-PYR. The hydrolyzed residues are neutralized with NaOH, and NaCl added. The solution is then partitioned with ethyl ether. Ethyl ether is removed by rotary evaporation, residues are redissolved in hexane:ethyl acetate (60:40, v:v) and subjected to silica gel column cleanup. The collected eluant is rotary evaporated and residues redissolved in methanol:water (4:1, v:v) for analysis of residues of 4'-OH-PYR and POP by HPLC using either UV or fluorescence (FLD) detection.

Method RM-33G-3: Residues of 2,5-OH-pyridine (including conjugates) are extracted from milk with ethyl acetate:methanol (1:1, v:v). Milk solids precipitate, the mixture is filtered and concentrated by rotary evaporation. Residues are hydrolyzed with 1 N HCl to release conjugates, and diluted for further cleanup through a Mega Bond Elut SCX column. The collected eluate is analyzed for residues of 2,5-OH-pyridine by HPLC/FLD.

Method RM-33T-1: Residues of pyriproxyfen are extracted from animal tissues with ethyl acetate:methanol (3:1, v:v). The extraction is repeated and the combined extracts rotary evaporated. Fats are removed by partitioning residues with ACN and hexane. The ethyl acetate phase is rotary evaporated and subjected to alumina column cleanup. The collected eluate is rotary evaporated and residues are redissolved in toluene for analysis of pyriproxyfen residues by GC/NPD.

Method RM-33T-2: Residues of 4'-OH-PYR (including conjugates) are extracted from animal tissues with methanol:water (3:1, v:v). The extract is vacuum filtered and the solvent rotary evaporated. The remaining aqueous residues are extracted with ethyl acetate, the solvent is

removed by rotary evaporation, and residues hydrolyzed with 1 N HCl (heated for 2 hours) to release conjugates. The acidic extract is washed with hexane to remove fats, and neutralized with NaOH. Sodium chloride was added and the residues partitioned into methylene chloride. The methylene chloride is rotary evaporated, the residues are dissolved in hexane:ethyl acetate (60:40, v:v), and subjected to silica gel column cleanup. The collected eluate is rotary evaporated and the residues redissolved in methanol:water (4:1, v:v) for analysis of 4'-OH-PYR residues by HPLC/UV.

Method RM-33T-3: Residues of POP (including conjugates) are extracted from liver and kidney with methanol:water (3:1, v:v). The solvent is removed by rotary evaporation, and the residues partitioned from water into ethyl acetate. The ethyl acetate was rotary evaporated, and residues partitioned with hexane and ACN to remove fats. The ACN phase was concentrated by rotary evaporation and residues hydrolyzed with 1 N HCl (heated 2 hours) to release conjugates. The extract is neutralized with NaOH, sodium chloride is added, and the residues partitioned into methylene chloride. The methylene chloride is rotary evaporated and the residues dissolved in hexane:ethyl acetate (60:40, v:v) and subjected to silica gel column cleanup. The collected eluate is rotary evaporated and the residues redissolved in methanol:water (4:1, v:v) and filtered for analysis of residues of POP by HPLC/FLD.

Method RM-33T-4: Residues of 2,5-OH-pyridine are extracted from liver and kidney with methanol:water (4:1, v:v). The extraction is repeated and the combined extract is partitioned with hexane to remove fats. The aqueous phase is concentrated by rotary evaporation, and the residues hydrolyzed with 1 N HCl. The hydrolyzed residues are diluted and subjected to further cleanup using a Mega Bond Elut C18 column and then Mega Bond Elut SCX column. The collected eluate is analyzed for residues of 2,5-OH-pyridine by HPLC/FLD.

The petitioner submitted method validation data for the above methods that were generated during method development. Untreated cow milk and liver were separately fortified with pyriproxyfen, 4'-OH-PYR, POP, and 2,5-OH-pyridine each at 0.020 and 0.10 ppm. Concurrent method recoveries were also included with the cattle feeding study. Untreated samples of each cattle matrix were separately fortified with pyriproxyfen, 4'-OH-PYR, POP, and 2,5-OH-pyridine at 0.02-0.20 ppm and analyzed using the methods described above. Method validation and concurrent recoveries are presented in Tables 7a and b, respectively.

Table 7a. Method validation recoveries from untreated cattle matrices separately fortified with pyriproxyfen, 4'-OH-PYR, and 2,5-OH-pyridine, and analyzed using GC/NPD and HPLC/UV or FLD methods.

Analyte (Method)	Fortification Level (ppm)	% Recoveries [a]
Milk		
Pyriproxyfen (RM-33G-2)	0.02, 0.10	90-103 (9)
POP (RM-33G-2)	0.02, 0.10	80-108 (9)
4'-OH-PYR (RM-33G-2)	0.02, 0.10	75-89 (9)
2,5-OH-pyridine (RM-33G-3)	0.02, 0.10	78-115 (10)
Liver		
Pyriproxyfen (RM-33T-1)	0.02, 0.10	95-103 (9)
POP (RM-33T-2)	0.02, 0.10	74-98 (9)
4'-OH-PYR (RM-33T-3)	0.02, 0.10	75-93 (9)
2,5-OH-pyridine (RM-33T-4)	0.02, 0.10	77-103 (9)

[a] Values in parentheses represent the number of samples for the given recoveries.

Table 7b. Concurrent method recoveries from untreated cattle matrices separately fortified with pyriproxyfen, 4'-OH-PYR, POP, and 2,5-OH-pyridine, and analyzed using GC/NPD and HPLC/UV or FLD methods.

Analyte (Method)	Fortification Level (ppm)	% Recoveries [a]
Milk		
Pyriproxyfen (RM-33G-2)	0.02, 0.10	71-104 (22)
4'-OH-PYR (RM-33G-2)	0.02, 0.10	70-105 (22)
POP (RM-33G-3)	0.02, 0.10	76-115 (22)
2,5-OH-Pyridine (RM-33G-3)	0.02, 0.10	66 (1); 78-120 (22); 129 (1)
Skim Milk		
Pyriproxyfen (RM-33G-2)	0.02, 0.10	90, 98 (2)
4'-OH-PYR (RM-33G-2)	0.02, 0.10	77, 94 (2)
POP (RM-33G-3)	0.02, 0.10	98, 108 (2)
2,5-OH-Pyridine (RM-33G-3)	0.02, 0.10	92, 102 (2)
Cream		
Pyriproxyfen (RM-33G-2)	0.02, 0.20	72-92 (4)
4'-OH-PYR (RM-33G-2)	0.04, 0.20	67 (1); 84 (1)
POP (RM-33G-3)	0.04, 0.20	67 (1); 92 (1)
2,5-OH-Pyridine (RM-33G-3)	0.04, 0.20	73, 74 (2)
Liver		
Pyriproxyfen (RM-33T-1)	0.02, 0.10	99 (2)
4'-OH-PYR (RM-33T-2)	0.02, 0.10	84, 88 (2)
POP (RM-33T-2)	0.02, 0.10	71-106 (4)
2,5-OH-Pyridine (RM-33T-3)	0.02, 0.10	84, 90 (2)

(continued; footnotes follow)

Table 7b (*continued*).

Analyte (Method)	Fortification Level (ppm)	% Recoveries [a]
Kidney		
Pyriproxyfen	0.02, 0.10	91, 97 (2)
4'-OH-PYR	0.02, 0.10	87 (2)
POP	0.02, 0.10	69 (1); 71-101 (3)
2,5-OH-Pyridine	0.02, 0.10	73-96 (4)
Muscle		
Pyriproxyfen	0.02, 0.10	87, 90 (2)
4'-OH-PYR	0.02, 0.10	77, 81 (2)
Fat		
Pyriproxyfen	0.02, 0.10	88, 94 (2)
4'-OH-PYR	0.02, 0.10	68 (1); 72 (1)

[a] Values in parentheses represent the number of samples for the given recoveries.

The petitioner also conducted an extraction efficiency study to validate the residue methods used to determine the concentrations of pyriproxyfen and its metabolites in milk and animal tissues.

MRID 44329509 Green, C.; Radke, H. J., (1997) Validation of the Extraction Efficiency of the Residue Analytical Methods for Pyriproxifen and Metabolites in Goat Meat and Milk: Lab Project Number: V-95-11604. Unpublished study prepared by Valent U.S.A. Corp. 204 p. {OPPTS 860.1340}.

Samples from a goat metabolism study (MRIDs 44036922 and 44036923; see PP#6F04737, CBTS No. 17440, DP Barcodes D228556, D228925, and D228926, J. Garbus, 06-MAY-1997) were re-analyzed using Methods RM-33G-2 and RM-33G-3 for milk, and Methods RM-33T-1, RM-33T-2, and RM-33T-4 for liver. The results of the radiochemical and residue method analyses are compared in Table 8.

Recoveries were also determined using the residue methods for samples of untreated milk and liver fortified separately with pyriproxyfen, 4'-OH-PYR, and 2,5-OH-pyridine at 0.020 and 0.10 ppm and analyzed concurrently with the treated samples. Concurrent method recoveries were 87-90% (pyriproxyfen), 81% (4'-OH-PYR), and 79-99% (2,5-OH-pyridine), in two samples each of milk; and 91-100% (pyriproxyfen), 57-66% (4'-OH-PYR), and 39-70% (2,5-OH-pyridine), in two samples each of liver. The petitioner notes that interference problems were encountered for the analysis of 2,5-OH-pyridine in both goat milk and liver, and

therefore, corrected the residue values from the treated matrices with the interference levels determined in the untreated sample.

Table 8. Comparison of radiochemical and residue method analyses of goat matrices from a goat metabolism study with [pyridyl-^{14}C]pyriproxyfen.

Analyte	Radiochemical Results (ppm) Based on Pyriproxyfen Equivalents	Radiochemical Results (ppm) Calculated Concentrations [a]	Residue Method Results (ppm) [b]
Milk			
Pyriproxyfen	0.003	0.003	<0.01, <0.01
4'-OH-PYR	0.041	0.043	0.04, 0.05
2,5-OH-Pyridine	0.033	0.011	0.02, 0.02 [c]
Liver			
Pyriproxyfen	0.004	0.004	<0.01, <0.01
4'-OH-PYR	0.078	0.082	0.048, 0.052 [d]
2,5-OH-Pyridine	0.058	0.020	0.012, 0.014 [e]

[a] Based on the molecular weight of the analyte relative to the molecular weight of pyriproxyfen.
[b] Determined in duplicate using the following residue methods: RM-33G-2 for pyriproxyfen and 4'-OH-PYR in milk; RM-33G-3 for 2,5-OH-pyridine in milk; RM-33T-1 for pyriproxyfen in liver; RM-33T-2 for 4'-OH-PYR in liver; and RM-33T-4 for 2,5-OH-pyridine in liver.
[c] Residues in the treated sample (0.042 ppm) were corrected for interferences in the control sample (0.021 ppm).
[d] Residue values were corrected for background in the control sample (0.003 ppm).
[e] Residue values were corrected for background in the control sample (0.026 ppm).

Study summary: Adequate descriptions and validation data have been submitted for the GC/NPD and HPLC methods for the determination of residues of pyriproxyfen and its metabolite 4'-OH-PYR in meat and milk, and residues of the metabolites POP and 2,5-OH-pyridine in milk, liver, and kidney. Although the petitioner indicated possible interferences which increased the LOD and LOQ to 0.02 and 0.04 ppm, respectively, in several matrices for the metabolites, the GC/NPD and HPLC methods are adequate for data collection of residues of pyriproxyfen in meat and milk.

OPPTS GLN 860.1360: Multiresidue Method

Multiresidue Testing data have previously been provided (PP#6F04737, DP Barcode D228556, J. Garbus, 06-MAY-1997) for pyriproxyfen and its metabolite PYPAC. Pyriproxyfen was recovered from fortified apple and cotton samples through protocol A, C, D, E, and F. The

metabolite PYPAC was tested with protocols A, B, C, and D. The results have been forwarded to FDA.

OPPTS GLN 860.1380: Storage Stability Data

Storage stability data - plant commodities: The RAC samples from the apple and pear field trials were frozen following collection, and shipped frozen to the analytical laboratory (Valent Technical Center, Dublin, CA). At the laboratory, fruit samples were macerated and preserved with 1 M ascorbic acid. The macerated samples were stored frozen (-20°C) until extraction and analysis. The total storage interval between harvest and analysis was 4-85 days (~0-3 months) for apple trials conducted in 1995-1996, and 194-224 days (~6-7 months) for the preliminary apple field trials conducted in 1994; and 27-64 days (~1-2 months) for pear trials conducted in 1995-1996, and 3-262 days (~0-9 months) for the preliminary pear field trials conducted in 1994.

The RAC samples from the walnut field trials were separated from the hulls and shells on the day of sampling, and frozen. In a single field trial, walnuts were dried in the field for one day before sampling and air-dried for three days indoors before shelling. Nutmeat samples were shipped frozen within 10 days of harvest, and shipped on dry ice via FedEx or by ACDS freezer truck to Valent Technical Center (Dublin, CA), where all samples were stored frozen (-20°C) until analysis. The total storage interval between harvest and analysis was 9-38 days (~0-1 month) for walnuts.

RAC samples from the apple processing study were shipped at cooled temperatures to Wm. J. Englar and Associates (Ephrata, WA) for processing. Samples were stored under refrigeration until processing; processing was completed within 72 hours of receipt. Processed samples were shipped frozen via overnight to Valent Technical Center for analysis. The RAC and processed samples were stored frozen (-20°C) at the laboratory until preparation for analysis. Juice and pomace samples were analyzed within 57 days (~2 months) of harvest.

To validate the storage intervals and conditions of RAC commodity samples collected from the field studies, and processed apple commodities from the processing study, the petitioner concurrently conducted storage stability studies. Untreated samples of walnuts, apples, apple pomace, and apple juice were fortified separately with pyriproxyfen and 4'-OH-PYR each at 0.10 ppm. Fortified samples were analyzed using the GC/FPD and HPLC methods initially and following frozen storage (-20°C) for the duration of the field trial studies. The results of the storage stability study are presented in Table 9.

Table 9. Recoveries of RAC and processed samples from the field trial and apple processing studies fortified with pyriproxyfen and 4'-OH-PYR and stored frozen (-20°C).

Commodity	Fortification Level (ppm)	Storage Interval (days)	Percent Recovery		
			Fresh Fortification	Storage Stability	Corrected Storage Stability [a]
Pyriproxyfen					
Apples	0.10	0	91, 94, 96	--	--
		40	93	61, 67	66, 72
		64	98	74, 78	76, 80
		89	88	54, 62	61, 70
		550	102	43, 56	42, 55
Apple, pomace	0.10	0	78, 83	--	--
		16	97	107, 109	111, 113
		50	81	63, 78	78, 96
Apple, juice	0.10	0	80, 83	--	--
		21	95	49, 55	52, 58
		35	94	24, 27	26, 29
		53	79	21, 31	26, 39
Walnuts	0.10	0	91, 94, 103	--	--
		30	98	90, 91	92, 93
		63	93	78, 93	84, 100
		90	96	84, 88	88, 92
4'-OH-PYR					
Apples	0.50	0	79, 80, 80	--	--
		107	85	77, 80	91, 94
Apple, pomace	0.10	0	74, 75	--	--
		34	72	52, 56	73, 78
		50	71	40, 43	56, 60
Apple, juice	0.10	0	86, 87	--	--
		21	90	84, 85	93, 94
		35	89	73, 74	82, 82
		53	83	63, 75	76, 90
Walnuts	0.10	0	83, 85, 85	--	--
		30	95	77, 83	81, 87
		63	97	70, 77	72, 79
		90	89	70, 76	79, 85

[a] Calculated by dividing the storage stability recovery by the fresh fortification recovery.

Conclusions: Adequate storage stability data are submitted in conjunction with the submitted field trials. The data demonstrate that residues of pyriproxyfen are fairly stable in frozen storage conditions for approximately 3 months in apples and walnuts, and 2 months in apple pomace. Residues of the metabolite 4'-OH-PYR are stable under frozen conditions for up to 3 months in/on apples and walnuts, 2 months in apple juice, and 1 month in apple pomace. Residues of pyriproxyfen appear to decline in apples following ~18 months storage (45-58%), and in apple juice following 21 days (42-48%), ~1 month (71-74%), and ~2 months (61-74%) of storage. Residues of 4'-OH-PYR appear to decline in apple pomace following ~2 months of storage (40-44%).

Samples from the submitted field trials were stored from harvest until analysis for 4-85 days (~0-3 months) for apples and pears, 9-38 days (~0-1 month) for walnuts, and 57 days (~2 months) for processed apple pomace and juice. The available frozen storage stability data support the storage intervals of the submitted field trials. We note that apple and pear samples from the preliminary field trials conducted in 1994 were stored for 3-262 days (~0-9 months). However, no additional storage stability data are required.

Storage Stability Data - Animal Commodities

For the cattle feeding study, samples of daily composited milk (a.m. and p.m. samples), separated cream and skimmed milk were stored frozen (-20°C) until extraction and analysis at the analytical laboratory; total storage intervals from collection to analysis were less than 11 days for whole milk, 6 days for skim milk, and 34 days for cream. Tissue samples were chilled with ice and water, homogenized, and immediately frozen at collection. Samples were shipped frozen to the analytical laboratory and stored frozen (-20°C) until analysis. The total storage intervals from collection to analysis were 10-24 days (< 1 month) for pyriproxyfen in liver, kidney, fat, and muscle; 46-72 days (~2 months) for 4'-OH-PYR in liver, kidney, fat, and muscle; 71-169 days (~2-6 months) for POP in liver and kidney; and 112-114 days (~4 months) for 2,5-OH-pyridine in liver and kidney. We note that fat and muscle samples from the feeding study were not analyzed for the metabolites, POP and 2,5-OH-pyridine.

The registrant conducted a storage stability study concurrent with the feeding study to demonstrate the stability of the residues of pyriproxyfen and its metabolites, 4'-OH-PYR, POP, and 2,5-OH-pyridine in cattle tissue matrices. A storage stability study was not initiated for milk, skim milk, and cream because these samples were analyzed within ~1 month. Duplicate untreated samples of liver, kidney, fat, and muscle from the cattle feeding study were separately fortified with pyriproxyfen and/or its metabolites, 4'-OH-PYR, POP, 2,5-OH-pyridine, 4'-SO_4-PYR, and POP-SO_4 each at 0.10 ppm. Concurrent method recoveries and recoveries of fortified samples following frozen storage are reported in Table 10.

Table 10. Storage stability recoveries of pyriproxyfen and its metabolites from fortified untreated samples of cattle commodities following frozen storage.

Cattle Matrix	Storage Interval	% Recovery [a]		
		Fresh Fortification	Storage Samples	Corrected Recoveries [b]
Pyriproxyfen				
Liver	0	86, 100, 103	--	--
	15	104	86, 90	82, 86
	32	105	79, 79	75, 75
Fat	0	90, 93	--	--
	14	96	86, 86	90, 90
	33	103	70, 78	68, 76
Muscle	0	91, 95	--	--
	16	88	71, 80	81, 91
	31	102	76, 82	75, 81
4'-OH-PYR				
Fat	0	76, 78	--	--
	13	70	66, 67	94, 96
	96	74	56, 67	76, 91
Muscle	0	75, 82, 84	--	--
	14	84	79, 80	94, 95
	30	75	72, 72	96, 96
	71	70	58, 64	82, 91
4'-SO_4-PYR				
Liver	0	81, 84	--	--
	15	70	76, 77	109, 111
	28	77	76, 77	98, 100
	57	66	67, 69	101, 105
POP-SO_4				
Liver	0	74, 76	--	--
	29	80	74, 81	92, 102
	51	67	63, 69	94, 102
	72	68	43, 59	63, 87
2,5-OH-Pyridine				
Kidney	0	86, 86	--	--
	22	83	65, 71	78, 86
	53	88	48, 60	55, 69
	119	70	16, 14	19, 22

[a] All samples were separately fortified with each analyte at 0.10 ppm.

[b] Recoveries of stored samples were corrected with the fresh fortification recovery.

Conclusions

The submitted storage stability data are adequate to support the storage intervals of the cattle feeding study. The submitted storage stability data indicate that residues of pyriproxyfen are stable under frozen conditions in liver, fat, and muscle for ~1 month; residues of 4'-OH-PYR are stable under frozen conditions in muscle for ~2 months and in fat for ~3 months; residues of 4'-SO_4-PYR are stable under frozen conditions in liver for ~2 months; and residues of POP-SO_4 are relatively stable in liver for ~2 months. Residues of 2,5-OH-pyridine were stable under frozen conditions in kidney for ~1 month but recoveries declined to 55-69% and 19-22% at the ~2 month and 4 month intervals, respectively.

Samples from the submitted feeding study were stored frozen for up to ~1 month for milk (pyriproxyfen, 4'-OH-PYR, POP, and 2,5-OH-pyridine); <1 month for pyriproxyfen in liver, kidney, fat, and muscle; ~2 months for 4'-OH-PYR in liver, kidney, fat, and muscle; ~2-6 months for POP in liver and kidney prior to analysis. As indicated above, liver and kidney samples treated with 2,5-OH-pyridine were stored for 112-114 days prior to analysis. The current storage stability data showing a 38-79% decline in residues over 53-119 days does not support the storage interval incurred by these samples. However, because the HED MARC (D250953, W. Donovan & W. Dykstra, 19-NOV-1998) determined that the 2,5-OH-pyridine metabolite is not a residue of concern in animal commodities, the lack of stability of this particular metabolite is not problematic. HED notes that fat and muscle samples from the feeding study were not analyzed for the metabolites, POP and 2,5-OH-pyridine. The submitted storage stability data support the storage intervals of the cattle feeding study; no additional storage stability data are required.

OPPTS GLN 860.1500: Crop Field Trials

Pome Fruits

Apples

Valent submitted the following data from 13 field trials depicting residues of pyriproxyfen in/on apples:

> MRID 44329505 Green, C. (1997) Magnitude of the Residues of Pyriproxyfen in/on Apples and Apple Processing Fractions: Lab Project Number: V-95-11119: V-11119-A: V-11119-B. Unpublished study prepared by Valent U.S.A. Corp. 1524 p. {OPPTS 860.1380,860.1500 and 860.1520}.

Thirteen trials were conducted in 1995-1996 in CA(1), CO(1), ID(1), MI(2), NY(1), NC(1), OR(1), PA(2), and WA(3). Table 11 outlines the various treatment schedules. In all thirteen trials, mature apples were harvested 43-45 days following the last of three broadcast

applications, with 13-105 day retreatment intervals, of the 0.86 lb/gal EC formulation at ~50 grams ai/A/application (0.11 lb ai/A/application) using ground airblast sprayer equipment in 95-106 gal/A of water. Total application rates were ~0.33 lb ai/A/season (1x the maximum proposed seasonal rate). Additional plots or harvest points were setup at various sites to demonstrate the decline of residues, 2x the maximum proposed seasonal rate, or applications with crop oil. In addition, two trials were conducted as a preliminary study in 1994 in NY and WA using varying application rates. An additional plot was not treated at each site.

Table 11. Treatment schedules of field trials conducted on apples.

Test Sites	PHI [a] (days)	Number of Applications	Application Rate (lb ai/A/ application)	Total Application Rate (lb ai/A)	GPA [b]
CA, CO, ID, MI(2), NC, OR, PA(2), and WA(3).	44-45	3	0.11	0.33	95-106
NY	38, 45, 52	3	0.11	0.33	99-101
WA	44	3	0.11	0.33	98-103 w/crop oil [c]
MI, NY, WA	45	3	0.22	0.66	96-102
NY, WA	98 or 143	2	0.11	0.22	204-296
	98 or 143	2	0.22	0.44	204-298
	28-29	3	0.11, 0.11, 0.066	0.286	200-296
	28-29	3	0.22, 0.22, 0.13	0.57	202-298

[a] Pre-harvest interval; days from the last application to harvest.
[b] Gallons per acre of water.
[c] Applications include one quart of crop oil/100 gal spray mixture.

Apples were harvested from control and treated trees, and frozen. Samples were analyzed for residues of pyriproxyfen and its metabolite, 4'-OH-PYR using GC/NPD and HPLC methods, respectively. Apparent residues of pyriproxyfen and its metabolite, 4'-OH-PYR were less than the LODs (<0.01 or <0.05 ppm) in/on all untreated apple samples. Residues of pyriproxyfen and its metabolite, 4'-OH-PYR in/on treated apples are reported in Table 12.

Table 12. Residues of pyriproxyfen and 4'-OH-PYR in/on apple fruits harvested following broadcast applications of the 0.86 lb ai/gal EC formulation.

Test Location (City, State)	PHI [a]	Uncorrected Residues (ppm)	
		Pyriproxyfen	4'-OH-PYR
Three broadcast applications of the 0.86 lb ai/gal EC formulation at 0.11 lb ai/A/application; 0.33 lb ai/A/season (1x the maximum proposed seasonal rate)			
Stockton, CA	43	0.07, 0.08	<0.01, <0.01
Austin, CO	45	0.11, 0.13	<0.05, <0.05
Payette, ID	45	0.07, 0.09	<0.01, <0.01
Conklin, MI	45	0.07, 0.13	<0.05, <0.05
Conklin, MI	45	0.08, 0.10	<0.01, <0.01
Alton, NY	38	0.07, 0.09	<0.01, <0.01
	45	0.08, 0.09	<0.01, <0.01
	52	0.05, 0.06	<0.01, <0.01
Oak Ridge, NC	45	0.05, 0.08	<0.05, <0.05
Parkdale, OR	45	0.07, 0.09	<0.01, <0.01
Hereford, PA	45	0.09, 0.13	<0.05, <0.05
Hereford, PA	45	0.14, 0.15	<0.01, <0.01
Ephrata, WA	45	0.14, **0.18**	<0.05, <0.05
Zillah, WA	45	0.06, 0.06	<0.01, <0.01
	45 [b]	0.07, 0.08	<0.01, <0.01
Zillah, WA	45	0.08, 0.09	<0.01, <0.01
Three broadcast applications of the 0.86 lb ai/gal EC formulation at 0.22 lb ai/A/application; 0.66 lb ai/A/season (2x the maximum proposed seasonal rate)			
Conklin, MI	45	0.13, 0.16	0.01, <0.01
Alton, NY	45	0.11, 0.13	<0.01, 0.01
Zillah, WA	45	0.14, 0.14	<0.01, 0.01
Two broadcast applications of the 0.86 lb ai/gal EC formulation at 0.11 lb ai/A/application; 0.22 lb ai/A/season (0.7x the maximum proposed seasonal rate)			
Alton, NY	98	<0.01, <0.01	<0.05, <0.05
Ephrata, WA	143	<0.01, <0.01	<0.05, <0.05
Two broadcast applications of the 0.86 lb ai/gal EC formulation at 0.22 lb ai/A/application; 0.44 lb ai/A/season (1.3x the maximum proposed seasonal rate)			
Alton, NY	98	<0.01, <0.01	<0.05, <0.05
Ephrata, WA	143	<0.01, <0.01	<0.05, <0.05

(continued; footnotes follow)

Table 12 (*continued*).

Test Location (City, State)	PHI [a]	Uncorrected Residues (ppm)	
		Pyriproxyfen	4'-OH-PYR
Three broadcast applications of the 0.86 lb ai/gal EC formulation at 0.11, 0.11, and 0.066 lb ai/A/application; 0.29 lb ai/A/season (0.9x the maximum proposed seasonal rate)			
Alton, NY	29	0.03, 0.03	<0.05, <0.05
Ephrata, WA	28	0.04, 0.04	<0.05, 0.05
Three broadcast applications of the 0.86 lb ai/gal EC formulation at 0.22, 0.22, and 0.13 lb ai/A/application; 0.57 lb ai/A/season (1.8x the maximum proposed seasonal rate)			
Alton, NY	29	0.06, 0.07	<0.05, <0.05
Ephrata, WA	28	0.10, 0.10	<0.05, 0.07

[a] Pre-harvest interval.
[b] One quart of crop oil was added per 100 gals. of spray mixture.

Pears

Valent submitted the following data from eight tests depicting residues of pyriproxyfen in/on pears:

MRID 44329512 Green, C. (1997) Magnitude of the Residues of Pyriproxyfen in/on Pears: Lab Project Number: V-95-11120: V-11120-A: V-11120-C. Unpublished study prepared by Valent U.S.A. Corp.883 p. {OPPTS 860.1500}.

Eight trials were conducted in 1994-1996 in CA(3), OR(3), PA(1), and WA(1). Table 13 outlines the various treatment schedules. In seven trials, mature pears were harvested 44-48 days following the last of three broadcast applications, with 14-91 day retreatment intervals, of the 0.86 lb ai/gal EC formulation at ~50 grams ai/A/application (0.11 lb ai/A/application) using ground airblast sprayer equipment in 96-108 gal/A of water. Total application rates were ~0.33 lb ai/A/season (1x the maximum proposed seasonal rate). Additional plots or harvest points were setup at various sites to demonstrate the decline of residues, 2x the maximum proposed seasonal rate, or applications with crop oil. In addition, a trial was conducted as a preliminary study in 1994 in OR using varying application rates. An additional plot was not treated at each site.

Table 13. Treatment schedules of field trials conducted on pears.

Test Sites	PHI [a] (days)	Number of Applications	Application Rate (lb ai/A/ application)	Total Application Rate (lb ai/A)	GPA [b]
CA(2), OR(2), PA, WA	44-48	3	0.11	0.33	96-108
CA	38, 45, 51	3	0.11	0.33	97-104
	45	3	0.22	0.66	100-104
OR	44	3	0.11	0.33	101-103 w/crop oil [c]
OR	143	2	0.11	0.22	221, 236
	143	2	0.22	0.44	218, 235
	28	3	0.12, 0.11, 0.064	0.294	227-236
	28	3	0.23, 0.22, 0.13	0.58	220-235

[a] Pre-harvest interval; days from the last application to harvest.
[b] Gallons per acre of water.
[c] 1 gal of crop oil/100 gal spray mixture for the first application, and with 1 quart of crop oil/100 gal spray mixture for the second and third applications.

Pears were harvested from control and treated trees, and frozen. Samples were analyzed for residues of pyriproxyfen and its metabolite, 4'-OH-PYR using GC/NPD and HPLC methods, respectively. Apparent residues of pyriproxyfen and its metabolite, 4'-OH-PYR were not reported for the untreated pear samples; however the petitioner did report that residues of pyriproxyfen and 4'-OH-PYR were less than the LODs (<0.01 or <0.05 ppm) in/on all untreated pear samples. Residues of pyriproxyfen and its metabolite, 4'-OH-PYR in/on treated pears are reported in Table 14.

Table 14. Residues of pyriproxyfen and 4'-OH-PYR in/on pear fruits harvested following broadcast applications of the 0.86 lb ai/gal EC formulation.

Test Location (City, State)	PHI [a]	Uncorrected Residues (ppm)	
		Pyriproxyfen	4'-OH-PYR
Three broadcast applications of the 0.86 lb ai/gal EC formulation at 0.11 lb ai/A/application; 0.33 lb ai/A/season (1x the maximum proposed seasonal rate)			
Watonsville, CA	38	0.01, 0.04	<0.01, <0.01
	45	0.03, 0.05	<0.01, <0.01
	51	0.04, 0.05	<0.01, <0.01

(continued; footnotes follow)

Table 14 (*continued*).

Test Location (City, State)	PHI [a]	Uncorrected Residues (ppm)	
		Pyriproxyfen	4'-OH-PYR
Live Oak, CA	45	0.02, 0.03	<0.01, <0.01
Walnut Grove, CA	45	0.03, 0.04	<0.01, <0.01
Odell, OR	45	0.04, 0.05	<0.05, <0.05
Parkdale, OR	44	0.07, 0.08	<0.01, <0.01
	44 [b]	0.08, **0.09**	<0.01, <0.01
Orefield, PA	48	0.02, 0.03	<0.05, <0.05
Zillah, WA	45	0.04, 0.04	<0.01, <0.01
Three broadcast applications of the 0.86 lb ai/gal EC formulation at 0.22 lb ai/A/application; 0.66 lb ai/A/season (2x the maximum proposed seasonal rate)			
Watsonville, CA	45	0.06, 0.07	<0.01, <0.01
Two broadcast applications of the 0.86 lb ai/gal EC formulation at 0.11 lb ai/A/application; 0.22 lb ai/A/season (0.7x the maximum proposed seasonal rate)			
Salem, OR	143	<0.01, <0.01	<0.05, <0.05
Two broadcast applications of the 0.86 lb ai/gal EC formulation at 0.22 lb ai/A/application; 0.44 lb ai/A/season (1.3x the maximum proposed seasonal rate)			
Salem, OR	143	<0.01, <0.01	<0.05, <0.05
Three broadcast applications of the 0.86 lb ai/gal EC formulation at 0.12, 0.11, and 0.064 lb ai/A/application; 0.29 lb ai/A/season (0.9x the maximum proposed seasonal rate)			
Salem, OR	28	0.01, 0.02	<0.05, <0.05
Three broadcast applications of the 0.86 lb ai/gal EC formulation at 0.23, 0.22, and 0.13 lb ai/A/application; 0.58 lb ai/A/season (1.8x the maximum proposed seasonal rate)			
Salem, OR	28	0.03, 0.03	<0.05, <0.05

[a] Pre-harvest interval.

[b] One gal. of crop oil was added per 100 gals. of spray mix for application 1, and one quart of crop oil was added per 100 gals. of spray mixture for applications 2 and 3.

The current guidance (OPPTS 860.1500, Tables 2 and 5) recommends that a minimum of 18 trials should be conducted in the major growing regions of the United States for the establishment of a tolerance for pome fruits. With respect to the location of field trials, the guidance recommends trials should be conducted as follows: (i) for pears, Regions 1 (1 trial), 10 (2 trials), and 11 (3 trials); and (ii) for apples, Regions 1 (3 trials), 2 (1 trial), 5 (2 trials), 9 (1 trial), 10 (1 trial), and 11 (4 trials). The submitted apple trials which reflect the proposed application rate were conducted in Regions 1 (2 trial), 2 (2 trials), 5 (2 trials), 9 (1 trial), 10 (1

trial), and 11 (6 trials including the 1 trial with crop oil additive). In addition, the eight preliminary apple trials were conducted in Regions 1 and 11. The submitted pear trials which reflect the proposed application rate were conducted in Regions 1 (1 trial), 10 (3 trials), and 11 (4 trials including the 1 trial with crop oil additive). In addition, the four preliminary pear trials were conducted in Region 11. The number and location of field trials is adequate to support the proposed uses on pome fruits.

Study Summary: The submitted field trial data on the representative crops of pome fruits are adequate. The submitted data indicate that residues of pyriproxyfen will not exceed the proposed tolerance for pome fruits (0.2 ppm) in/on samples harvested 43-48 days following the last of three broadcast applications of the 0.86 lb/gal EC formulation at 0.11 lb ai/A/application (0.33 lb ai/A/season; 1x the maximum proposed seasonal rate). Residues of pyriproxyfen and 4'-OH-PYR were 0.05-0.18 and <0.05 ppm, respectively, in/on 28 samples of apples and 0.01-0.09 and <0.05 ppm, respectively, in/on 16 samples of pears treated as described above. In addition, the trials conducted with crop oil additive demonstrated no significant differences in residue levels from applications with or without crop oil additive.

Residues of pyriproxyfen remained relatively consistent in/on apples and pears harvested 38, 45, and 51 or 52 days following the treatment schedule described above (1x the maximum proposed seasonal rate), demonstrating that residues do not appear to decline or accumulate with respect to the harvest interval. Residues of pyriproxyfen were 0.07-0.09 ppm in apples and 0.01-0.04 ppm in pears harvested at the 38-day PHI; 0.08-0.09 ppm in apples, and 0.03-0.05 ppm in pears harvested at the 45-day PHI; and 0.05-0.06 ppm in apples, and 0.04-0.05 ppm in pears harvested at the 51- or 52-day PHI. Residues of 4'-OH-PYR were less than the LOD (<0.05 ppm) at all PHIs sampled.

Based on the residue values obtained from apple and pear samples harvested following treatment at the proposed maximum use rates, the proposed tolerance level of 0.2 ppm for residues of pyriproxyfen in/on pome fruits is appropriate.

Walnuts

Valent submitted the following data from four tests depicting residues of pyriproxyfen in/on walnuts:

MRID 44329511 Pensyl, J. (1997) Magnitude of the Residues of Pyriproxyfen and its Degradates in Walnuts: Lab Project Number: VP-11467:VP-96-11467: V-11467-A. Unpublished study prepared by Valent U.S.A. Corp. 381 p. {OPPTS 860.1500}.

Four trials were conducted in 1996 in CA. Mature walnuts were harvested 20-21 days following the last of three broadcast applications, with 14 day retreatment intervals, of the 0.86 lb/gal EC formulation at ~50 grams ai/A/application (0.11 lb ai/A/application) using ground airblast sprayer equipment in 97-109 gal/A of water. Total application rates were 150-162 grams ai/A/season (0.33-0.36 lb ai/A/season; 1x the maximum proposed seasonal rate). An additional plot was not treated at each site.

Walnuts were harvested (knocked down) from control and treated trees, and one control and duplicate treated samples were collected on the harvest day from each test. Walnut samples from a single trial were dried in the field for one day before collection. On the day of sampling, walnut nutmeats were separated from the hulls and shelled by hand or using mechanical shellers, and frozen. Walnuts dried in the field for one day before sampling were air-dried for three days indoors before shelling. Samples were analyzed for residues of pyriproxyfen and its metabolite, 4'-OH-PYR using GC/NPD and HPLC methods, respectively.

Residues of pyriproxyfen and its metabolite, 4'-OH-PYR were each less than the LOD (<0.01 ppm) in/on four samples each of untreated walnut nutmeat. Residues of pyriproxyfen and its metabolite, 4'-OH-PYR were each less than the LOD (<0.01 ppm) in/on eight samples of walnut nutmeat harvested 20-21 days following the last of three broadcast applications of the 0.86 lb/gal EC at ~50 grams ai/A/application (0.11 lb ai/A/ application). We note that a single treated nutmeat sample originally had detectable levels of 4'-OH-PYR (0.01 ppm); however, residues were below the LOD (<0.01 ppm) when the sample was re-analyzed using the alternative fluorescence HPLC detector. Residues of pyriproxyfen and 4'-OH-PYR were below the LOQ (<0.02 ppm) in all samples.

A total of 4 field trials were conducted with walnuts in Region 10. The number and location of field trials is adequate to support the proposed uses on walnuts.

Study Summary: The submitted field trial data on walnuts are adequate. The submitted data indicate that residues of pyriproxyfen will not exceed the proposed tolerance for walnuts (0.02 ppm) in/on samples harvested 21 days following the last of three broadcast applications of the 0.86 lb/gal EC formulation at ~50 grams ai/A/application (0.33 lb ai/A/season; 1x the maximum proposed seasonal rate). Residues of pyriproxyfen and 4'-OH-PYR in/on eight samples of walnuts treated as described above were each less than the LOQ (<0.02 ppm).

Based on the nondetectable residue values obtained from samples harvested following treatment at the proposed maximum use rates, the proposed tolerance level of 0.02 ppm for residues of pyriproxyfen in/on walnuts is appropriate.

OPPTS GLN 860.1520: Processed Food/Feed

Apples

Valent submitted the following data depicting the potential for concentration of pyriproxyfen residues in the processed commodities of apples:

> MRID 44329505 Green, C. (1997) Magnitude of the Residues of Pyriproxyfen in/on Apples and Apple Processing Fractions: Lab Project Number: V-95-11119: V-11119-A: V-11119-B. Unpublished study prepared by Valent U.S.A. Corp. 1524 p. {OPPTS 860.1380.860.1500 and 860.1520}.

In two tests conducted in MI and WA, mature apples were harvested 45 days following the last of three broadcast applications, with 15-68 day retreatment intervals, of the 0.86 lb ai/gal EC formulation at 0.22 lb ai/A/application (0.66 lb ai/A/season; 2x the maximum proposed seasonal rate).

Control and bulk treated samples were collected (hand picked) from the designated test plots. The harvested composite samples were shipped at cooled temperatures on the day of harvest to Wm. J. Englar and Associates (Ephrata, WA) for processing. Samples were stored refrigerated until processing; the fruit was stored for a maximum of 72 hours prior to processing. Samples were processed according to simulated commercial procedures into apple pomace and juice. A brief description of the processing procedure follows. Apples were washed with cold water and the washed apples crushed in a hammermill. The crushed apple pulp was heated with low steam until the temperature reached 40-50°C. Pectic enzyme was added to the pulp and after 2 hours the enzyme treated pulp was pressed in a fruit press and wet pomace and juice collected. The juice was filtered to remove any coarse solids. The moisture content of the wet pomace was determined at 53-54%. Processed samples were shipped frozen to Valent Technical Center (Dublin, CA) for analysis.

Residues of pyriproxyfen and 4'-OH-PYR in/on treated and untreated apples and apple processed commodities were determined using GC and HPLC methods. Apparent residues of pyriproxyfen and 4'-OH-PYR were each less than the LOD (<0.01 ppm) in/on two samples of untreated apples and one sample each of apple pomace and juice processed from untreated apples. Residues in treated samples are presented in Table 15.

Table 15 Residues of pyriproxyfen and 4'-OH-PYR in the processed commodities of apples harvested 45-47 days following the last of three broadcast applications at 2x the proposed maximum seasonal rate.

Trial Site	Substrate	Residues, ppm		Concentration/Reduction Factor	
		Pyriproxyfen	4'-OH-PYR	Pyriproxyfen	4'-OH-PYR
Conklin, MI	Apple, whole fruit	0.16	0.02	--	--
	Apple, juice	<0.01	<0.01	--	--
	Apple, pomace	0.70	0.08	4.4x	4x
Zillah, WA	Apple, whole fruit	0.17	0.01	--	--
	Apple, juice	<0.01	<0.01	--	--
	Apple, pomace	0.92	0.06	5.4x	6x

Study Summary: The submitted apple processing data are adequate. The data indicate that residues of pyriproxyfen concentrated 4.4-5.4x and residues of 4'-OH-PYR concentrated 4-6x in pomace processed from apples bearing detectable residues. No concentration of residues was observed in juice processed from treated apples.

Based on the available field trial data, the HAFT for apples harvested 45 days following treatment at the maximum proposed seasonal application rate (0.33 lb ai/A) is 0.16 ppm for residues of pyriproxyfen (4'-OH-PYR residues were <0.05 ppm). The maximum pyriproxyfen residues expected in apple pomace, based on the HAFT and the average concentration factor 4.9x, would be 0.78 ppm. Based on the highest expected residues, the proposed tolerance of 0.8 ppm for pyriproxyfen residues in/on apple wet pomace is adequate.

OPPTS GLN 860.1480: Meat, Milk, Poultry and Eggs

Ruminants

Valent has submitted the following feeding study on lactating dairy cows to determine the distribution of pyriproxyfen and pyriproxyfen metabolite residues in edible tissues and milk:

> MRID 44329510 Green, C. (1997) Residues in Meat and Milk from Dairy Cows Fed Pyriproxyfen: Lab Project Number: V-96-11445: RM-33G-2: RM-33G-3. Unpublished study prepared by Valent U.S.A. Corp. and Bio-Life Associates, Ltd. 695 p. {OPPTS 860.1480}.

The in-life phase of the study was conducted by Bio-Life Associates (Neillsville, WI). Following a 19-day acclimation period, nine dairy cows were orally dosed daily following the evening milking for 28 consecutive days with pyriproxyfen (three cows per dosing group). The test substance was encapsulated in gelatin capsules and administered using a balling gun. Animals received Purina Milk Chow Special (a pre-mixed feed ration); the feed consumed was measured and used to calculate the required test substance during the study. Three additional

cows were not treated and served as control animals. Control animals received empty gelatin capsules.

Using proposed tolerances for animal feed items, the calculated maximum theoretical dietary burdens for beef and dairy cattle are 1.69 and 1.29 ppm, respectively (Table 16). Based on the dietary burdens, the dosing levels of 3, 9, and 30 ppm represent ~2x, 5x, and 18x the maximum theoretical dietary burden to beef cattle, and ~2x, 7x, and 23x the maximum theoretical dietary burden to dairy cattle.

Table 16. Calculation of the maximum theoretical dietary burden of pyriproxyfen to beef and dairy cattle.

Feed Commodity	Estimated Tolerance, ppm	% Dry Matter [a]	Beef Cattle		Dairy Cattle	
			% of Diet	Burden, ppm	% of Diet	Burden, ppm
Apple, pomace, wet	0.8	40	40	0.80	20	0.40
Cotton gin byproducts[b]	2.0	90	20	0.44	20	0.44
Citrus, pulp[c]	1.0	91	20	0.22	20	0.22
Almond hulls[d]	2.0	90	10	0.22	10	0.22
Cotton, seed[b]	0.05	88	10	0.0057	25	0.014
TOTAL			100	1.69	95	1.29

[a] Table 1, OPPTS GLN 860.1000.
[b] From PP#6F04737, D241303 & D228499, W. Donovan, W. Dykstra, and B. Tarplee, 27-FEB-1998.
[c] From 98CA0011, D243702, M. Lamont, W. Dykstra, and B. Tarplee, 25-MAR-1998.
[d] From 98CA0041, D249441, W. Donovan, W. Dykstra, and M. Christian, 26-OCT-1998.

Animals were observed throughout the study for clinical abnormalities. Feed consumption and milk production were recorded daily, and animals were weighed prior to dosing and on days 14, and 28 (sacrifice). There was no apparent effect of pyriproxyfen dosing on overall animal health, feed consumption, or milk production.

The cows were milked twice daily (a.m. and p.m.), and milk samples were collected. Milk samples for a given study day consisted of the pooled a.m. and p.m. milk sample (pooled in proportion to production). On day 24 of the study, the composited milk sample was separated by centrifugation into cream and skim milk. All milk samples were frozen and transferred to the analytical laboratory (Valent Technical Center, Dublin, CA) where samples were frozen (-20°C) until analysis.

Control and treated cattle were sacrificed after 28 days of dosing. Animals were sacrificed within 24 hours of the final dose. Samples of fat (composite of perinephric, abdominal, and subcutaneous), muscle (composite of hind-quarter, pectoral, and abductor), liver, and kidney were collected. Tissue samples were chilled with ice and water, homogenized, and

immediately frozen. Samples were shipped frozen on dry ice to Valent Technical Center for analysis. Samples were analyzed for residues of pyriproxyfen and its metabolites 4'-OH-PYR and POP using the GC/NPD and HPLC methods described under the Residue Analytical Method Section.

For analysis of whole milk, single samples from each cow were analyzed from study days 1, 2, 7, 10, and 14 for each dose group; additional whole milk samples were analyzed from study days -1, 4, 17, 21, 24, and 28 for the 9 and/or 30 ppm dose groups. Skim milk samples were analyzed from study day 24 for the 30 ppm dose group, and cream samples were analyzed for the 9 and 30 ppm dose groups. For analysis of residues in tissues, single samples of each matrix were analyzed for each cow in each dose group. The results from the analyses of milk fractions and tissues are presented in Table 17.

Table 17. Residues of pyriproxyfen and its metabolites in dairy cow matrices following oral administration of pyriproxyfen at 3, 9, and 30 ppm over 28 consecutive days.

Dose Level (ppm)	Dosing or Sample Day [a]	Residue (ppm) [b]			
		Pyriproxyfen	4'-OH-PYR	POP	2,5-OH-Pyridine
Whole Milk					
3	1	--	--	--	<0.01
	2	--	--	--	<0.01
	7	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
	10	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
	14	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
9	1	--		--	<0.01
	2	--	--	--	<0.01
	7	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
	10	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
	14	<0.01, <0.01, <0.01	<0.01, <0.01, 0.01 [c]	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
30	-1	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
	1	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
	2	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
	4	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
	7	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
	10	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
	14	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
	17	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
	21	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
	24	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
	28	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01

(continued; footnotes follow)

Table 17 (*continued*).

Dose Level (ppm)	Dosing or Sample Day [a]	Residue (ppm) [b]			
		Pyriproxyfen	4'-OH-PYR	POP	2,5-OH-Pyridine
Skim Milk					
30	24	<0.01, <0.01, <0.01	<0.02, <0.02, <0.02 [d]	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
Cream					
9	24	<0.01, <0.01, <0.01	--	--	--
30	24	0.01, 0.01, 0.02	<0.02, <0.02, <0.02	<0.02, <0.02, <0.02	<0.02, <0.02, <0.02
Liver					
9	28	<0.01, <0.01, <0.01	--		
30	28	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01
Kidney					
9	28	<0.01, <0.01, <0.01	--	--	--
30	28	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	<0.02, <0.02, <0.02	0.02, 0.01, 0.02
Fat					
3	28	<0.01, <0.01, <0.01	--	--	--
9	28	0.01, 0.02, 0.03	--	--	--
30	28	0.05, 0.06, 0.07	<0.01, <0.01, <0.01	--	--
Muscle					
30	28	<0.01, <0.01, <0.01	<0.01, <0.01, <0.01	--	--

[a] Dosing ceased on Day 28, and animals were sacrificed within 24 hours of sacrifice.
[b] Residue values reported each represent a single sample.
[c] Petitioner suspects lab error because all other samples were <0.01 ppm.
[d] Detection limit was increased as 0.015 ppm was found in both control and treated samples.

Following oral dosing of cattle with pyriproxyfen at 3, 9, and 30 ppm for 28 consecutive days, nondetectable (<0.01 ppm) residues of pyriproxyfen and its metabolites 4'-OH-PYR, POP, and 2,5-OH-pyridine were observed in whole milk (3, 9, and 30 ppm dose levels), and liver (30 ppm dose level). Detectable pyriproxyfen residues (0.01-0.02 ppm) and nondetectable residues (<0.01 or <0.02 ppm) of 4'-OH-PYR, POP, and 2,5-OH-pyridine were present in cream at the 30 ppm dose level. Dectectable 2,5-OH-pyridine residue levels were also found in liver samples collected on day 28 at the 30 ppm dose level. Fat and muscle were only analyzed for pyriproxyfen and 4'-OH-PYR. Nondetectable residues (<0.01 ppm) of pyriproxyfen and 4'-OH-PYR were observed in muscle at the 30 ppm dosing level; detectable pyriproxyfen residues (0.05-0.07 ppm) and residues of 4'-OH-PYR below the LOD (<0.01 ppm) were present in fat at the 30 ppm dosing level. Residues of pyriproxyfen were nondetectable (<0.01 ppm) in fat at the 3 ppm dosing level and detectable (0.01-0.03 ppm) at the 9 ppm dosing level; 4'-OH-PYR was not analyzed for in these samples. The highest residue levels of pyriproxyfen (0.05-0.07 ppm) were observed in fat at the highest dose level (30 ppm; 18x the maximum theoretical dietary burden for beef cattle).

Conclusions

Typically, tolerances are required on all animal commodities having detectable residue levels at a 10x dosing rate or below. For the computed MTDB of 1.69 ppm in beef cattle, this would include the 3 and 9 ppm dosing levels. The only commodity having detectable pyriproxyfen residues at these levels was fat: 0.01 - 0.03 ppm. Since the MTDB calculation is based on a nutritionally unbalanced diet and includes contributions from some animal feed items that are used only regionally, HED will not require the establishment of pyriproxyfen tolerances in fat at this time. However, should future new uses include additional animal feed items, tolerances on animal commodities will be needed.

Poultry

There are no poultry feed items associated with pome fruits and walnuts. Therefore, no additional secondary residues are expected to occur in poultry eggs, fat, meat, and meat byproducts as a result of the additional proposed uses. Based on the submitted cattle feeding study and the previously submitted discussion (PP#6F04737, DP Barcode D228556, J. Garbus, 06-MAY-1997), no secondary residues are expected in meat, milk, poultry, and eggs from the proposed uses.

OPPTS GLNs 860.1850 and 860.1900: Confined/Field Accumulation in Rotational Crops

Because pome fruits and walnuts are not rotated, no rotational crop (confined or field) studies are required to support these proposed uses for pyriproxyfen.

Codex Harmonization

An International Residue Limits Status sheet is attached. There are no Codex, Canadian, or Mexican tolerances for residues of pyriproxyfen in/on pome fruits or walnuts; thus, harmonization is not an issue. Pyriproxyfen is scheduled as a new compound for JMPR review (both toxicology and chemistry) in 1999.

Attachment 1: EPA memoranda cited in this review.
Attachment 2: International Residue Limit Status sheet for pyriproxyfen.

cc with Attachments: PP#7F04882, RAB1 File, W.H. Donovan, O. Odiott
RDI: G. Kramer (07-DEC-1998), RAB1 Chemists (03-DEC-1998), M. Morrow (07-DEC-1998)
W. Donovan:CM#2:RM804E:305-7330:07-DEC-1999

EPA MEMORANDA CITED IN THIS REVIEW

CBTS No.:	17440
DP Barcode:	D228556, D228925, and D228926
Subject:	PP#6F4737. Pyriproxyfen on Cotton. Evaluation of Analytical Methods, Field Trial, and Processing Residue Data.
From:	J. Garbus
To:	K. Boyle
Dated:	06-MAY-1997
MRID(s):	44036901-44036904, 44036918-44036920, 44036922-44036930, 44037201, and 4437204.

DP Barcode:	D241303, D228499
Subject:	PP#6F04737. Pyriproxyfen on cotton. HED Risk Assessment.
From:	W. Donovan, W. Dykstra, B. Tarplee
To:	S. Lewis, J. Tavano
Dated:	27-FEB-1998

DP Barcode:	D243702
Subject:	ID#98CA0011. Section 18 Exemption for the use of Pyriproxyfen on Citrus in California.
From:	M. Lamont, W. Dykstra, B. Tarplee
To:	A. Beard, R. Forrest
Dated:	25-MAR-1998

DP Barcode:	D249441
Subject:	ID#98CA0041. Section 18 Exemption for the use of Pyriproxyfen on Almonds to Combat Fire Ants in California.
From:	W. Donovan, W. Dykstra, M. Christian
To:	A. Beard, R. Forrest
Dated:	26-OCT-1998

DP Barcode:	D250953
Subject:	Pyriproxyfen. Results of the Metabolism Assesment Review committee Meeting Held on 10-NOV-1998.
From:	W. Donovan, W. Dykstra
To:	G. Kramer
Dated:	19-NOV-1998

Attachment 2

INTERNATIONAL RESIDUE LIMIT STATUS

Chemical Name:	Common Name: pyriproxyfen	X□ Proposed tolerance □ Reevaluated tolerance □ Other	Date: 12/01/98

Codex Status (Maximum Residue Limits)	U. S. Tolerances
X□ No Codex proposal step 6 or above □ No Codex proposal step 6 or above for the crops requested	Petition Number: 7F04882 DP Barcode: Other Identifier:
Residue definition (step 8/CXL): N/A	Reviewer/Branch: W. Donovan/RAB1
	Residue definition: pyriproxyfen

Crop (s)	MRL (mg/kg)	Crop(s)	Tolerance (ppm)
		pome fruit	0.2
		walnuts	0.02
		apple pomace, wet	0.8

Limits for Canada	Limits for Mexico
X□ No Limits □ No Limits for the crops requested	X□ No Limits □ No Limits for the crops requested
Residue definition: N/A	Residue definition: N/A

Crop(s)	MRL (mg/kg)	Crop(s)	MRL (mg/kg)

Notes/Special Instructions: Codex, Scheduled as a new chemical in 1999 (tox and residue)

13544

R137540

Chemical: Pyriproxyfen

PC Code:
129032

HED File Code: ~~11000 Chemistry Reviews~~ 14500 Pesticides Chem

Memo Date: 12/7/1998

File ID: DPD238190

Accession #: 000-00-0113

HED Records Reference Center
1/31/2007